



**PREPARATION AND CHARACTERIZATION
OF INCLUSION COMPOUNDS OF
PHARMACEUTICALS WITH
CYCLODEXTRIN**

ABSTRACT

THESIS

SUBMITTED FOR THE AWARD OF THE DEGREE OF

Doctor of Philosophy

IN

CHEMISTRY

BY

ARTI MAHESHWARI

DEPARTMENT OF CHEMISTRY
ALIGARH MUSLIM UNIVERSITY
ALIGARH (INDIA)

2006



The thesis entitled **“Preparation and Characterization of Inclusion Compounds of Pharmaceuticals with Cyclodextrin”** is comprised of an introduction, a general discussion and ¹H NMR spectroscopic study of inclusion complexes of five pharmaceutical compounds with β-cyclodextrin (β-CD).

The introduction describes a brief historical background, structures of CDs and its derivatives, examples of various industrial applications of CDs inclusion complexes with the main emphasis in the area of pharmaceuticals, chiral recognition by the CDs and the forces involved in the host-guest complexation. The general discussion deals with the use of ¹H NMR spectroscopy in the study of CD-inclusion complexes. There are five chapters on the study of inclusion complexes of pharmaceutical compounds with β-CD. The objective of the study was to characterize the structures of the complexes formed in aqueous solution because the use of these complexes for pharmaceutical purposes requires the establishment of their structures.

CDs are doughnut shaped molecules composed of glucose units joined through α-(1-4) linkage. Three most common, industrially important, α, β, and γ-CDs are composed of 6, 7 and 8 glucose units, respectively. The primary hydroxyl groups (n) are located at the narrow rim while wider rim is lined with secondary hydroxyl groups (2n). The outer surface of the CDs is highly hydrophilic due the presence of large number of hydroxyl groups while the CD cavity is relatively hydrophobic. Considering the presence of 18(α-CD), 21(β-CD) and 24(γ-CD) substitutable hydroxyl groups and involving cleavage of either O-H, C-O or C-C bonds, the number of possible derivatives of CDs are unlimited. Functionalization of CDs can concern complete or partial functionalization of hydroxyls.

THEESIS

By virtue of their shape and hydrophobic nature of cavity, CD_s accommodate a variety of hydrophobic molecules, or part of it, inside their cavity through non-covalent interactions to form inclusion complexes. Inclusion of the guest into the CD cavity results in altered physicochemical properties of the guest, like solubility, stability and volatility etc., which have found numerous applications in the field of pharmaceuticals.

Biological systems are largely constructed from chiral molecules such as L-amino acids and D-sugars. In this highly chiral environment it is not surprising that drugs which possess asymmetric center/s exhibit a high degree of stereoselectivity in their interactions with biological macromolecules. It may be highly desirable to have all the activity in one enantiomer of the drug so as to avoid the unwanted activity and/or toxicity that may reside in the antipode but examples where this has been proven to be true are uncommon. The separation of enantiomers and their characterization are, therefore, of great importance for the pharmaceutical industry. CDs are chiral molecules and exhibit chiral recognition, i.e., they differentiate between enantiomeric species, forming diastereomeric complexes. Enantioselectivity of recognition is a thermodynamic quantity correlating in some way with the separation factor of enantiomers in separation techniques. It must be noted that among presently applied chiral selectors only CDs are effectively used in all enantioseparation techniques. A better understanding of the inclusion complex formation and the chiral recognition mechanism of CDs is, therefore, a subject of great importance.

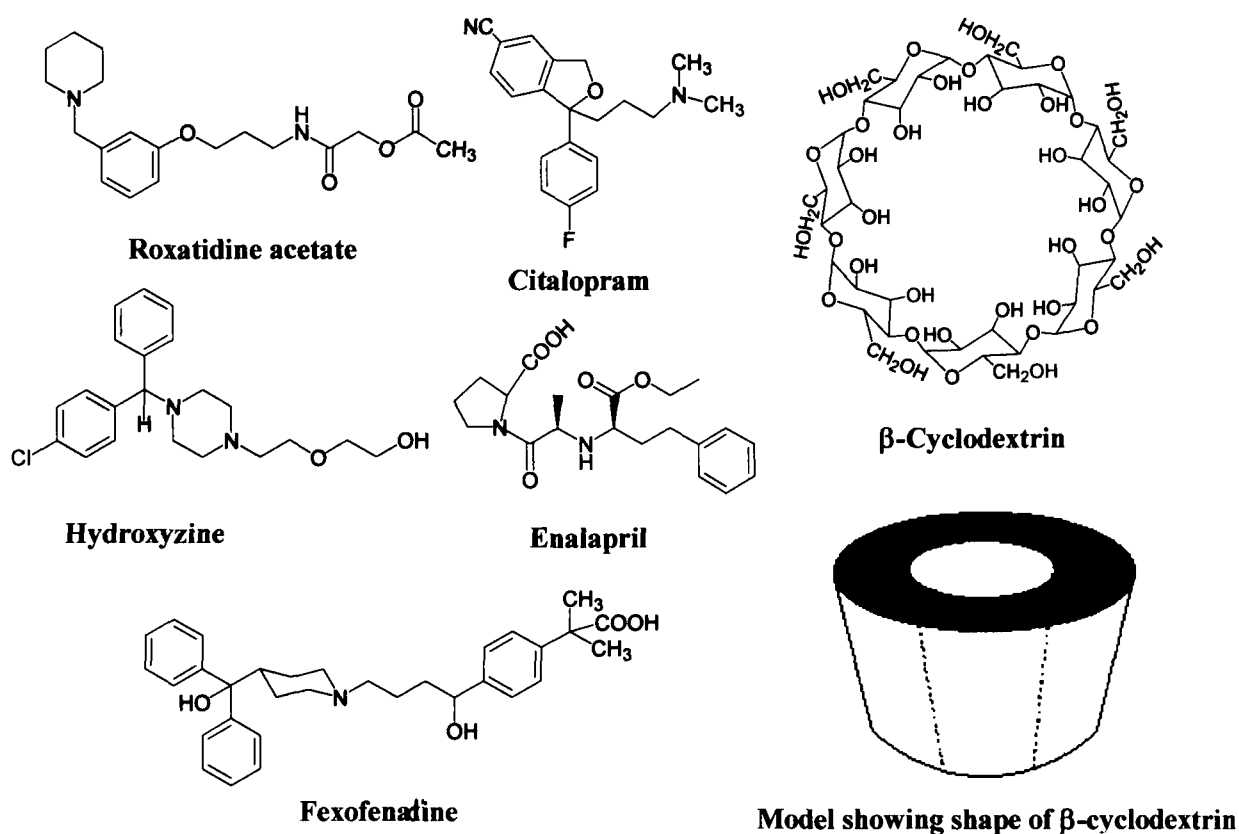
Multiple forces involved in guest-CD interactions make understanding of guest binding and chiral recognition mechanism by CDs extremely difficult. The understanding of the driving forces in the inclusion complexation of CDs is fundamentally important not only for CD

chemistry but also for supramolecular chemistry as a whole. A large number of studies have been carried out on the subject and have been reviewed earlier. Nevertheless, it is often claimed that the driving forces leading to CD complexation still remain a controversial subject.

NMR spectroscopy is an important technique to study inclusion complexes. The formation of an inclusion complex between a guest and CD results in the chemical shift changes in both the host and guest protons. The highfield shift changes in the CD cavity protons confirm the penetration of the guest into the CD cavity. The guest protons generally show downfield shift changes upon complexation but all the protons and not only the part included in the cavity display these changes. Sometimes highfield shift changes in the guest proton signals are also observed. Determination of stoichiometry of the complex is required, for any further investigation of the complex, which is usually achieved by NMR titrations studies. Nuclear Overhauser Effect (NOE) studies can be used to fully characterize the inclusion complex. It gives information about the part of the guest included inside the CD cavity, the mode of penetration, i.e. either from narrower or wider rim side, the depth of penetration and orientation of the guest.

^1H NMR spectroscopic studies of complexation of roxatidine acetate hydrochloride, citalopram, hydroxyzine hydrochloride, enalapril maleate and fexofenadine hydrochloride with β -CD were carried out. In all the cases, the chemical shift changes in the proton resonances of studied compounds and β -CDs were observed in the spectra of mixtures of pharmaceutical compound and β -CD compared to pure component. β -CD cavity protons, namely H-3' and H-5', showed significant highfield shift changes in the presence of drug

while other protons generally showed insignificant chemical shift changes. Stoichiometry and overall binding constant of the inclusion complexes were determined by ^1H NMR titration methods. The mode of penetration and orientation of the guest molecule into the β -CD cavity were established by ROESY/NOESY spectral data in all the studied cases.



Roxatidine acetate hydrochloride (RAH) forms one 1:1 RAH- β -CD complex in aqueous solution by the shallow penetration of the aromatic ring, due to the presence of two bulky groups in the meta position, from wider rim side of the β -CD cavity. The structure for the RAH- β -CD complex has been proposed.

(RS)-Citalopram (CT) contains a F-substituted and a cyano-substituted aromatic rings. It forms 1:1 inclusion complexes with β -CD in aqueous solution involving both the aromatic rings. The modes of penetration of both the rings were identical i.e. from wider rim side of the cavity. There was chiral discrimination by the β -CD between the two enantiomeric forms and the mechanism of chiral recognition seems to be different modes of binding of two enantiomers in the complexes involving cyano-containing ring. The structures of all the complexes formed in solution between citalopram and β -CD have been characterized.

(RS)-Hydroxyzine hydrochloride (HYZ) has two aromatic rings and both of these rings were confirmed to be involved in the inclusion complex formation with β -CD. Four 1:1 HYZ- β -CD complexes were characterized, two formed by the entry of the phenyl ring and two by the penetration of the p-chlorophenyl ring from both the sides of the β -CD cavity. The structures for all the complexes present in solution have been proposed. There was no clear evidence for the chiral discrimination by the β -CD between two enantiomers.

It was confirmed by the detailed ^1H NMR spectroscopic study that (SSS)-enalapril maleate (EN) exists in two geometrical forms in solution and an unambiguous ^1H NMR assignment of the signals, which appeared separately for two isomers, was made. Enalapril maleate formed 2:1 EN- β -CD complexes in aqueous solution. It was confirmed that both, the aromatic as well as heterocyclic, rings are involved in complexation. ROESY confirmed that β -CD cavity is occupied by an aromatic and a heterocyclic ring at the same time. It was also evidenced that aromatic ring approached the cavity from

narrower rim side while heterocyclic entered from wider rim side. Four 2:1 (EN- β -CD) diastereomeric complexes must, therefore, be present in solution. There was a significant change observed in the ratio of two geometrical isomers in one sample in the presence of β -CD which suggests some catalysis by the β -CD but this needs further confirmation.

(RS)-Fexofenadine (FFN) has two phenyl and one para substituted aromatic rings. Complexes involving all the aromatic rings were found to be present in solution. The modes of penetration of phenyl and p-substituted aromatic rings into the β -CD cavity were different. The phenyl ring entered the cavity from wider rim side while p-substituted ring approached the cavity from narrower rim side. It was found that FFN forms 1:1 inclusion complexes when the concentration of β -CD is less than or equal to that of FFN ($[\beta\text{-CD}] \leq [\text{FFN}]$) and as the concentration of β -CD increases the 1:2 FFN- β -CD complexes are favoured. The structures for all the possible inclusion complexes present in solution have been proposed. There was some evidence of chiral discrimination between two enantiomers by β -CD.

THESIS



**PREPARATION AND CHARACTERIZATION
OF INCLUSION COMPOUNDS OF
PHARMACEUTICALS WITH
CYCLODEXTRIN**

THESIS

SUBMITTED FOR THE AWARD OF THE DEGREE OF

Doctor of Philosophy

IN

CHEMISTRY

BY

ARTI MAHESHWARI

**DEPARTMENT OF CHEMISTRY
ALIGARH MUSLIM UNIVERSITY
ALIGARH (INDIA)**

2006



T6804

DEDICATION

To my parents and family

for their guidance, support, love and enthusiasm

without these things this thesis could not have been possible.

Dr. S. Mashhood Ali




Department of Chemistry
Aligarh Muslim University
Aligarh-202 002, India
Ph: 09719060155
E-mail: smashhoodali@yahoo.com

Dated...15 April, 2006

CERTIFICATE

Certified that the work embodied in this thesis entitled "**Preparation and Characterization of Inclusion Compounds of Pharmaceuticals with Cyclodextrin**", has been carried out by **ARTI MAHESHWARI** under my supervision and the same has not been submitted for a degree elsewhere.


(Syed Mashhood Ali)
Supervisor

Acknowledgements

There are lots of people I would like to thank for a huge variety of reasons.

It is difficult to overstate my gratitude to my Ph.D. supervisor, Dr. Syed Mashhood Ali. I could not have imagined having a better advisor and mentor for my Ph.D., and without his knowledge, common-sense, perceptiveness and cracking-of-the-whip, I would not have accomplished this task. Throughout my thesis-writing period, he provided encouragement, sound advice, good teaching, good company, and lots of good ideas that I would have missed without him.

I am highly obliged to Prof. Kabiruddin, Chairman, Department of Chemistry, Aligarh Muslim University, Aligarh for providing me research facilities.

I must express my thanks to all my friends/colleagues particularly Fahmeena, Santosh, Zainab, Pooja, Sarita, Nishat, Sheetal, for being the surrogate family all the time and for their continued moral support and help throughout the tenure of this work whenever needed. I am also thankful to Azra aunty, Hamza and Huzefa for their kind support.

I owe a great deal to Professors P. De Waard, Netherlands, Steve W. Homans, UK, John C. Edwards, Danbury, Grant Collins, Australia, Kye Chun Nam, Korea, Du Li, USA, Zhaogue Zhang, China, Michael G. Organ, Canada, Mamoru Koketsu, Japan, all of whom were very kind to help me in obtaining NMR data.

I feel overwhelmed to acknowledge the assistance of R. L. Fine Chemicals, Nebulae Health Care Ltd., Surya Pharmaceutical Ltd, P. I. Pharmaceuticals, India and Geertrui Haest, Cerestar Cargill, Belgium, who were very generous to send gift samples of pure drugs and cyclodextrins.

Finally, I am forever indebted to my parents, Mr. Prem Kumar Maheshwari and Mrs. Rajbala Maheshwari, brother Chirag, sister Pooja, Jiju Pankaj and Prakhar for their understanding, endless patience and encouragement when it was most required. I am indebted to Shalini, Yashu, Neha, Mukta, Varsha, Trapti, Yadhu, for providing a stimulating and fun environment at my home to learn and grow and for all the emotional support, comradery, entertainment and caring they provided.

Most of all thanks to GOD, the Divine, who continues to make the impossible possible.

Arti Maheshwari
Arti Maheshwari

CONTENTS

Aim and Objective	
1. Introduction	1
1.1 Historical Background	2
1.2 Industrial Applications of Cyclodextrins	7
A. General Applications of Cyclodextrins	9
B. Pharmaceutical Applications of Cyclodextrins	14
1.3 Chiral Recognition by Cyclodextrins	30
1.4 Forces Involved in Cyclodextrin Complexation	44
2. General Discussion	56
3. ¹H NMR Spectroscopic Study of Complexation of Roxatidine Acetate Hydrochloride with β-Cyclodextrin	76
4. ¹H NMR Spectroscopic Study of Complexation of Citalopram with β-Cyclodextrin	104
5. ¹H NMR Spectroscopic Study of Complexation of Hydroxyzine Hydrochloride with β-Cyclodextrin	128
6. ¹H NMR Spectroscopic Study of Complexation of Enalapril Maleate with β-Cyclodextrin	144
7. ¹H NMR Spectroscopic Study of Complexation of Fexofenadine Hydrochloride with β-Cyclodextrin	164
8. Conclusion and Suggestion for Further Work	<i>Annexure</i>
9. References	183
10. List of Publications	203

AIM AND OBJECTIVE

The objective of the work embodied in this thesis was to study the complexation of various pharmaceutical compounds with β -cyclodextrin in aqueous solution. Complexation of a pharmaceutical compound with cyclodextrins, to form a host-guest complex, results in altered various physicochemical properties of the guest, like solubility, stability, volatility and masking of undesirable properties etc., which are desirable for their use as pharmaceuticals. Moreover, these host-guest complexes are considered as new entities and are required to be characterized for their approval as a drug. CDs are chiral molecules and differentiate between enantiomeric species. Enantioselectivity of recognition is related in some way to the separation factor of enantiomers in separation techniques. The separation of enantiomers of a racemic drug is of great importance to the pharmaceutical industry because in a racemic drug one enantiomer is usually unwanted. NMR spectroscopy is a very useful tool to understand the mechanism of chiral recognition and a better understanding of the subject will help in the use of cyclodextrins as chiral selectors in various separation techniques.

1. Historical Background

Supramolecular chemistry is the discipline which involves all kinds of interactions where covalent bonds are not formed between the interacting species. The majority of these interactions are of the host-guest type. Among all the potential hosts, the cyclodextrins (CDs) are the most important.

In 1891 Villiers isolated a crystalline substance, which later proved to be a cyclodextrin (CD), by digesting starch with *Bacillus amylobactor* and determined its composition to be $(C_6H_{10}O_5)_2 \cdot 3H_2O$. He named this substance as “cellulosine” simply because it resembled cellulose.¹ Twelve years later, Schardinger observed the same substance and was able to isolate and examine it. Schardinger was the first to extensively study CDs and for this reason CDs are also referred to as Schardinger’s dextrins.² However, it was not until the 1930s that Freudenberg and coworkers³ established the structure of CDs, based partly on their own studies and partly on the observations published by Miekeley,⁴ and others.⁵ In the 1950s, the chemical process for the production of CDs was thoroughly investigated by French and coworkers⁶ and the existence of larger CDs was confirmed. Also, during this time Cramer et. al.⁷ first began to uncover the CDs potential as complex forming agents. They examined the ability of CDs to complex with a variety of drug molecules, and noted the stabilization, volatility reduction, and solubility changes that occurred as a result of complex formation of drugs with CDs. They subsequently obtained a patent in 1953 that covered practically all of the most important aspects of applications of CDs in drug formulations.⁸ The number of patents and papers on CDs have increased exponentially since then. In 1981 the first International Symposium on Cyclodextrins⁹ was organized. Since then onward, symposia

have been held every second year and presentations have increased both in quantity and quality. Moreover, while in 1970 the price of 1 Kg of β -CD was around US\$ 2000, and it was available only as a rare fine chemical, it is a relatively inexpensive material and an important industrial commodity now in many ways. Also, α -, β - and γ -CDs, as well as several of their derivatives are produced industrially and used in various chromatographic methods, or are studied as potential drug carriers, stabilizers, catalysts, etc.

Structure of Cyclodextrins

The three most common α -, β - and γ -CDs, consisting of 6, 7 and 8 glucopyranosyl units, respectively, are linked through α -(1 \rightarrow 4) glycosidic bonds (Fig. 1). The internal diameter of the three CDs increases with the number of glucose units while the height remains constant at 7.9 Å. The glucose units are in the rigid 4C_1 chair conformation giving them a toroidal shape with all the secondary hydroxyl groups, O(2)-H and O(3)-H (2n), located on the wider rim while all the primary hydroxyl groups, O(6)-H (n), on the narrower rim. The primary hydroxyls on the narrow side of the cavity can rotate, thus partially blocking the cavity, in contrast to the secondary hydroxyls, which are attached by relatively rigid chains and thus cannot rotate.

The interior of the cavity is lined with (from the secondary hydroxyl rim inwards) a row of CH groups (the C-3 carbons), then a row of glycosidic oxygen, and then a row of C-5 CH groups. Intramolecular hydrogen bonds O(3)-H \cdots O(2)-H or O(3) \cdots H-O(2) exist between the secondary hydroxyl groups of adjacent glucose units, forming a complete secondary belt in β -CD. This hydrogen bond belt is incomplete in α -CD molecule due to the presence of one

glucose unit in distorted position. The γ -CD has a non-planar and more flexible structure. The non-bonding electron pairs, of the glycosidic oxygen atom, are directed towards inside of the cavity producing a high electron density and giving the cavity some Lewis base character. The interior of the cavity is hydrophobic while the exterior is hydrophilic.¹⁰⁻¹² Table 1 gives some of the physical properties of the α -, β - and γ -CDs.

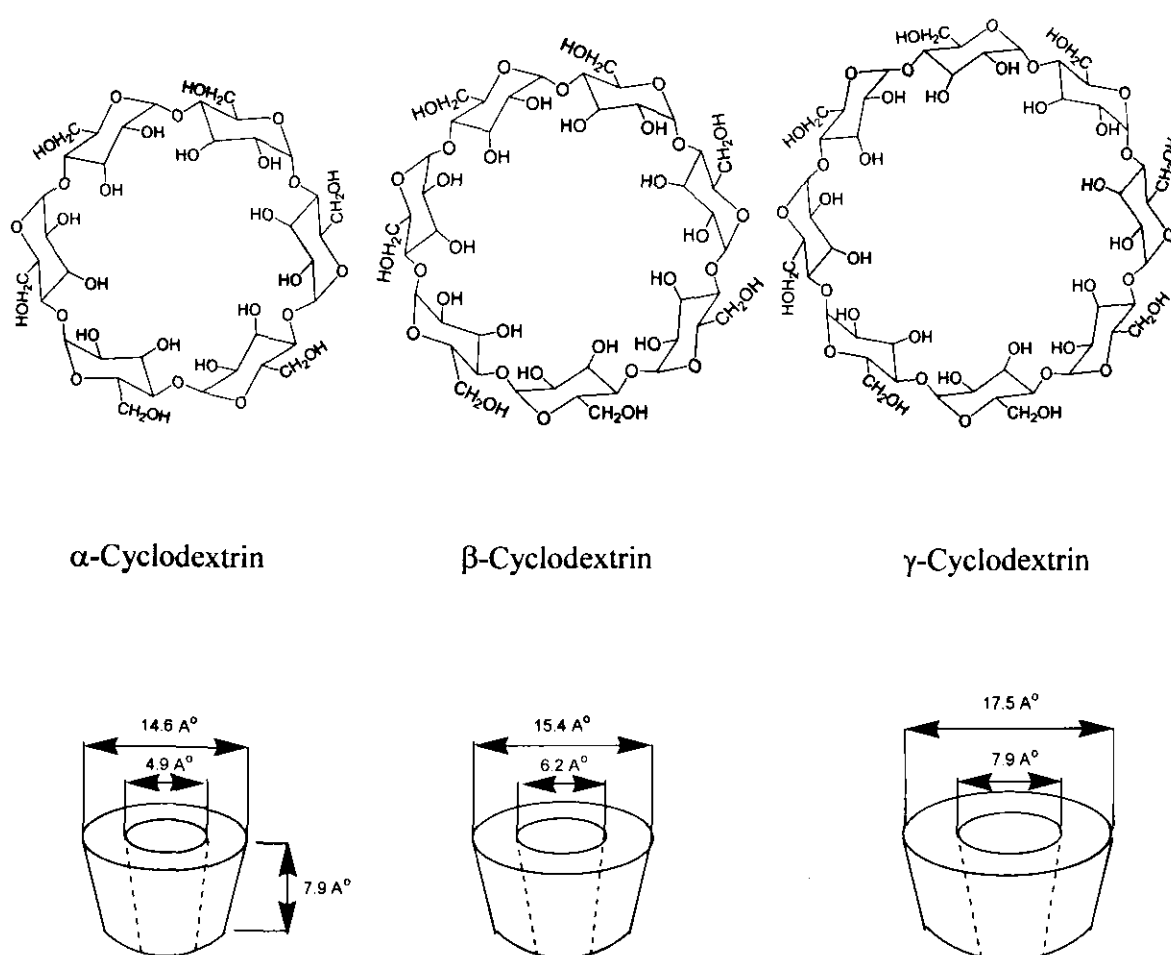


Fig. 1 Structures and sizes of cyclodextrins.

Table 1 Characteristics of α -, β -, and γ -CDs.

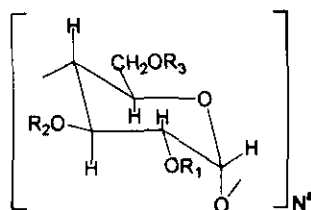
Property	α	β	γ
No. of glucose units	6	7	8
Appearance	White crystalline powder	White crystalline powder	White crystalline powder
Molecular weight	972	1135	1297
Water solubility (%)	14.5	1.85	23.2
Cavity diameter, Å	4.9	6.2	7.9

Cyclodextrin Derivatives

Today, a number of derivatives of CDs are formed and being used in various ways. Various functional groups can covalently be attached to the hydroxyl groups.^{13, 14} The complexation behaviour of CDs is altered when the hydroxyl groups are modified.

Considering the presence of 18(α -CD), 21(β -CD) and 24(γ -CD) substitutable hydroxyl groups and involving cleavage of either O-H, C-O or C-C bonds, the number of possible derivatives of CDs are unlimited. All the known CDs derivatives can be classified as carriers (solubilizers, stabilizer) for biologically active substances, enzyme models, separating agents (for chromatography) and catalysts and additives (as detergents, viscosity modifiers etc.).^{11, 12} Actually, methylated CDs (randomly methylated- β -CD), hydroxyalkylated-CDs (hydroxypropyl- β -CD and hydroxypropyl- γ -CD) and branched CDs (glycosyl- and maltosyl- β -CD) are produced due to cost/benefit ratio. Complexation with such functionalized CDs can also take place in organic solvents since many of the functionalized CDs are soluble in organic solvents. Functionalization of CDs can concern complete sets of hydroxyls or partial functionalization. Commercial derivatives of cyclodextrins are shown in Table 2.

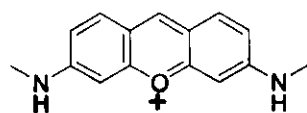
Table 2 Chemical structures of cyclodextrin derivatives.



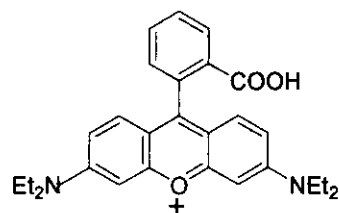
Compound	R ₁	R ₂	R ₃
Hydrophilic Derivatives			
Methylated cyclodextrins			
3-Mono-O-methylcyclodextrins	H	CH ₃	H
2, 6-Di-O-methylcyclodextrins	CH ₃	H	CH ₃
2, 3, 6-Tri-O-methylcyclodextrins	CH ₃	CH ₃	CH ₃
Randomly methylated cyclodextrins	R ₁ , R ₂ , R ₃ = H or CH ₃		
Hydroxylalkylated cyclodextrins			
2-Hydroxyethylcyclodextrins	R ₁ , R ₂ , R ₃ = H or CH ₂ CH ₂ OH		
2-Hydroxypropylcyclodextrin	R ₁ , R ₂ , R ₃ = H or CH ₂ CH(OH)CH ₃		
3-Hydroxypropylcyclodextrin	R ₁ , R ₂ , R ₃ = H or CH ₂ CH ₂ CH ₂ OH		
2, 3-Dihydroxypropylcyclodextrin	R ₁ , R ₂ , R ₃ = H or CH ₂ CH(OH)CH ₂ OH		
Branched cyclodextrins			
6-O-Glucosylcyclodextrins	H	H	glucose
6-O-Maltosylcyclodextrins	H	H	maltose
2, 6-O-Dimaltosylcyclodextrins	maltose	H	maltose
Hydrophobic Derivatives			
Alkylated cyclodextrins			
2, 6-Di-O-ethylcyclodextrins	C ₂ H ₅	H	C ₂ H ₅
2, 3, 6-Tri-O-ethylcyclodextrins	C ₂ H ₅	C ₂ H ₅	C ₂ H ₅
Acylated cyclodextrins			
2, 3-Di-O-hexanoylcyclodextrins	COC ₅ H ₁₁	COC ₅ H ₁₁	H
2, 3, 6-Tri-O-acetylcyclodextrins	COCH ₃	COCH ₃	COCH ₃
2, 3, 6-Tri-O-propanoylcyclodextrins	COC ₂ H ₅	COC ₂ H ₅	COC ₂ H ₅
2, 3, 6-Tri-O-butanoylcyclodextrins	COC ₃ H ₇	COC ₃ H ₇	COC ₃ H ₇
2, 3, 6-Tri-O-valerylcyclodextrins	COC ₄ H ₉	COC ₄ H ₉	COC ₄ H ₉
2, 3, 6-Tri-O-hexanoylcyclodextrins	COC ₅ H ₁₁	COC ₅ H ₁₁	COC ₅ H ₁₁
2, 3, 6-Tri-O-octanoylcyclodextrins	COC ₇ H ₁₅	COC ₇ H ₁₅	COC ₇ H ₁₅
Ionizable Derivatives			
Anionic cyclodextrins			
6-O-(Carboxymethyl)cyclodextrins	H	H	CH ₂ COONa
6-O-(Carboxymethyl)-	C ₂ H ₅	C ₂ H ₅	CH ₂ COONa
2,3-di-O-ethylcyclodextrins	R ₁ , R ₂ , R ₃ = H or SO ₃ Na		
Cyclodextrin sulfates	R ₁ , R ₂ , R ₃ = H or (CH ₂) ₄ SO ₃ Na		
Sulfobutylcyclodextrins			
^a N = 6, α-CDs; N = 7, β-CDs; N = 8, γ-CDs; N = 9, δ-CDs.			

2. Industrial Applications of Cyclodextrins

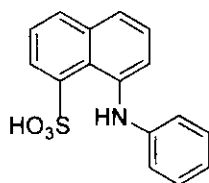
Today in industry, CDs and their derivatives are incorporated into a variety of items. CDs ability to form inclusion complexes with a variety of compounds has found usefulness in a multitude of areas. CDs are already in use in cosmetics, chewing gum, detergents, shampoo and toothpaste and for the formulation of drugs. Due to their nontoxic and non-hazardous nature, CDs have found applications in usually all kinds of everyday products. At present CDs have general approval for use as food additives in Japan and Hungary and for specific applications in France and Denmark. This section will include a broad but brief introduction into the different ways CDs and their derivatives are being used today in industry due to their ability to accommodate guest molecules in their cavity. The industrial applications of CDs have been divided into two parts: general applications and pharmaceuticals applications. Fig. 2 shows the structures of some compounds that have been utilized recently for the formation of complexes involving β -CD derivatives.



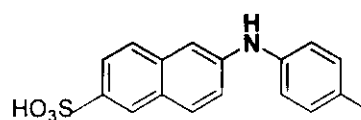
Acridine Red



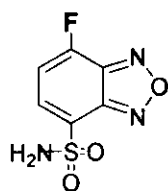
Rhodamine B



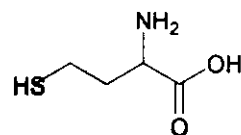
8-Anilino-1-naphthalenesulfonic acid



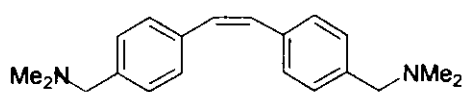
2-(p-toluidino)naphthalene-6-sulfonic acid



4-aminosulfonyl-7-fluoro-2,1,3-benzoxadiazole



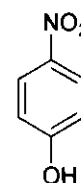
Homocysteine



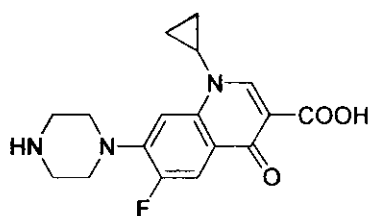
(Z)-4,4-Bis(dimethylaminomethyl)stilbene



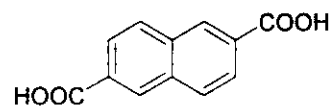
2,5-Norbornadiene



4-Nitrophenol



Ciprofloxacin



2,6-Naphthalenedicarboxylic acid

Fig. 2 Names and structures of some typical pharmaceutical compounds utilized recently in the formation of host-guest complexes with β -CD derivatives.

A. General Applications of Cyclodextrins

Solubility

The solubility of a compound can be altered by complexation with a CD.¹⁵ The guest molecule in the cavity of the CD does not come in contact with the solvent. The outer surface of the CD interacts with the solvent, which contributes to the solubility of the complex and not the portion of the guest included in the CD cavity. Modification of the hydroxyl group of the outer surface of the CD markedly affects the solubility properties. Substitution of the hydroxyl groups with hydroxypropyl, carboxymethyl, tertiary amine or quaternary amine increases water solubility upto 60% or more. On the other hand, substitution of –OH groups with aliphatic groups such as hexyl or acetyl results in increased solubility in organic solvents.

The cloudiness and bitter taste in the orange juice was discovered due to the presence of hesperidine. Addition of β -CD resulted in clearing the syrup and masking the bitter flavour of hesperidine.¹⁶ Triterpenes which are water insoluble, such as oleanolic acid, ursolic acid and gederogenin, could be solubilized upto 400 $\mu\text{g/ml}$ or more using CDs.¹⁷ Phenylalanine, tyrosine and tryptophan units of the peptides complex well with CDs resulting in the formation of CD-peptide complex. Ovine growth hormone is water insoluble, except at high pH such as 11.5, which is not useful for pharmacological purposes. It can be solubilized at pH 7.5 to 8.5 when complexed with hydroxypropyl β -CD.¹⁸ Some plant oils cause a rash and irritation when they come in contact with skin. Washing can result in spreading of the oils and increased irritation. These oils can be removed from the skin by complexation with

cyclodextrins especially γ -CD and hydroxypropyl β -CD.¹⁹ Large lipids such as ear wax can also be solubilized by complexation with γ -CD.²⁰

Glycolic acid is used for superficial peeling of the skin but is irritating to the skin. When applied complexed with cyclodextrins, it was found to be non-irritating and due to prolonged release of glycolic acid there was increase in efficiency of exfoliating action and cellular removal.²¹

CDs have also been used for dyeing fabrics. Using CDs, more dye was absorbed on the fabric reducing the amount of dye left in the waste water. The solubility of the dye in water also increased and no auxiliaries were needed to solubilize the dye. A 3-fold increase in the binding of the florescent dye to polyester fiber was achieved using hydrophobic tosyl derivatives of β -CD.²²

Viscosity of water-based paints during their manufacture can be controlled using CDs.²³ Thickeners are added to paints to impart desired viscosity properties. By complexing CD with the thickener during the manufacture of the paints, the viscosity is reduced, making mixing easier. Viscosity is restored upon addition of paint components which displace the thickener from the cavity of the CD to give the paints its desired viscosity properties.

Process Aids

CDs have found invaluable roles as processing aids in industry. CD has been used in the removal of cholesterol from animal products.²⁴ In one case, aqueous solution of CD was stirred with heated lard and tallow from which cholesterol was to be removed. The CD forms

complex with cholesterol which is insoluble in water or fat. The complex is removed by filtration or centrifugation. The CD-cholesterol complex is then suspended in water and heated which results in decomposition of complex causing CD to be solubilized in water and cholesterol to float on the surface. This way upto 80% of cholesterol has been removed from some treated material and CDs and cholesterol are obtained as pure products. CD complexation of cholesterol has also been found useful in reducing dietary cholesterol intake.²⁵

Fruit and vegetable juices were treated with a β -CD polymer to remove polyphenolics responsible for enzymatic browning.²⁶ Naringen and limonene, the bitter components of citrus fruits, have been removed using a polymer of β -CD.²⁷ Organic compounds need to be solubilized in water for bioconversions and therefore surfactants or solvents are used but these can be used in limited quantities because they affect the organism as well. By the use of methylated β -CD, a 6-fold increase in the bioconversion rate of podophyllotoxin was achieved compared to bioconversion in the absence of CD.²⁸

Stabilization

Compounds capable of making complexes with CDs are stabilized as a result of complexation, because they cannot interact with other molecules. Moreover, there is steric hindrance, which prevents approach by a molecule to the exposed portion of the guest at the open cavities. Unsaturated fatty acids, for example in fish oil, are easily oxidized resulting in unpleasant taste and odor. These oils can be protected from oxidation by complexation with CD.²⁹ Peroxyacids are used as bleaching agents and their thermal stability and storage are a

matter of concern. Complexation with CDs not only improves storage and thermal stability but odour is also reduced and complex is stable without using dilutants.³⁰ Explosives can also be stabilized by complexation with CDs.³¹

Masking of the Guest Effects

A guest molecule in the CD complex gets isolated, resulting in the masking of its various properties.³² This technique has been used in masking the unwanted flavour in food and skin irritation effects. Lotions have been developed in which a fragrant but irritation causing component is complexed with CD in order to limit its direct physical contact with skin but still allowing evolution of the desired fragrance. The irritating or toxic effects of insecticides can also be reduced or eliminated by complexation with CDs. Azinophos-methyl forms an odourless complex with β -CD.³³ No toxic effects were observed when the insecticide was administered dermally at a dose of 4000 mg/kg as CD-complex while free insecticide shows dermal toxicity at a dose of 18 mg/kg.

Reduction of Volatility

Reduction of volatility and controlled release of chemicals are other important applications of CDs. Menthol, for example, forms an odourless complex with β -CD which can be dried at 100 °C while free menthol will be completely volatilized under these conditions. The release of fragrance from laundry dryer sheets can be controlled by complexing the fragrance with CDs.^{34, 35}

Moreover, all the components of the fragrance have different volatility and some of the more highly volatile components are easily lost changing the composition of the fragrance. However, when these components are complexed with CDs, their volatility is reduced so that the character of the fragrance is not changed. CDs can also be used to prevent odour in the skin tanning and hair care preparations. A dry powder of CD can be used in products such as menstrual products, diapers, tissues, paper towels etc.³⁶ making them effective scavengers of unwanted odours. Perfumes can also be complexed to CDs to be released upon dissolution of the complex and displacement by odiferous compounds.

Directing of Chemical Reactions

CDs are also used for the mediation of specific organic reactions. The formation of host/guest complex, not only alter the parameters such as solubility, but inclusion of the part of the guest molecule into the CD-cavity offers significant steric shielding. This concept has been utilized in many ways for highly selective syntheses.³⁷ 2, 6-Naphthalenedicarboxylic acid is an important monomer for liquid crystalline polymer synthesis. Synthesis of 2, 6-naphthalenedicarboxylic acid is difficult due to several reactive sites on the naphthalene ring leading to a range of products. It was found that when naphthalene was first complexed with CD then the yield of the desired 2, 6-product was greatly improved.³⁸ The host CD sterically shielded the undesirable reaction sites on the naphthalene ring.

B. Pharmaceutical Applications of Cyclodextrins

Of all the industrial applications of CDs, a large portion of their uses is devoted to their applications in the field of pharmaceuticals. The most common pharmaceutical applications of CDs are to enhance the solubility, stability and bioavailability of drug molecules. Each CD has its own ability to form inclusion complexes with specific guests, an ability which depends on a proper fit of the guest molecule within the hydrophobic CD cavity. At present, some 30 or so drug formulations are approved and marketed worldwide as CD complexes, generally natural CD complexes (Table 3). The structures of some of the pharmaceutical compounds marketed as CD complexes are shown in Fig. 3.

The principal advantage of natural CDs are their (1) well defined chemical structures yielding many potential sites for chemical modification (2) easy availability and low cost (3) low toxicity and pharmacological activity (4) some water solubility and (5) protection of included drug molecules from biodegradation. However, natural CDs have relatively low solubility, both in water and organic solvents which limit their uses in pharmaceutical formulations. β -CD, the most common natural CD, has 21 hydroxyl groups, i. e. 7 primary and 14 secondary, which are available as starting points for structural modifications and so a variety of CD derivatives have been prepared so as to extend the physicochemical properties and inclusion capacity of the parent host molecule. Typical examples of the pharmaceutically useful β -CD derivatives are listed in Table 2, classified into hydrophilic, hydrophobic and ionic derivatives.

When mono- or disaccharides are attached to one or two primary hydroxyl groups of CD through 2,6-glycosidic linkage, their water solubility markedly increases: the solubility of 6-O-glucosyl- β -CD, 6-O-maltosyl- β -CD in water is over 50% (w/v) at 25 °C.⁴⁹ The complexation ability of branched CDs against hydrophobic guests is comparable to parent CDs and decreases only slightly with increase in the glucose number and the degree of substitution. However, the solubilizing ability of branched CDs is much greater than that of parent CDs. Branched CDs have higher affinity towards steroidal drugs such as progesterone, testosterone, dehydrocholic acid, digitoxin etc.^{49, 50} Due to their high solubilizing effect, branched CDs may be useful for parenteral preparations such as injections because of their weak hemolytic activity and high bioadaptability.

The solubility of CDs can significantly be affected by water miscible cosolutes and cosolvents. For example, urea is known to increase solubility of a variety of polar and non-polar organic solutes in water. The solubilities of β - and γ -CD were significantly enhanced in the presence of urea while that of α -CD decreased. Such solubility changes were observed for cosolvents such as alcohols, acetonitrile, dimethyl sulfoxide, dimethyl formamide, acetic acid and ethylene glycol etc.⁵¹⁻⁵³

The cosolubilization method has been found useful for the preparation of solid 2-hydroxypropylated CD complexes with unstable drugs such as steroids, peptides and antibiotics. The synergetic effect of CD derivatives (methylated, hydroxyalkylated, carboxymethylated and branched forms) and water soluble polymers (polyvinyl pyrrolidone, hydroxypropylmethyl cellulose and carboxymethyl cellulose) on the solubility of various poorly water soluble drugs were studied. It was found that the solubilizing effect of

2-hydroxypropyl- β -CD is enhanced on an average by 27% by carboxymethyl cellulose and 49% by polyvinyl pyrrolidone.^{54, 55} Table 4 summarizes the solubility enhancement studies of variety of pharmaceutical compounds in the presence of CDs.

Table 4 Examples of CD enhanced solubility of pharmaceuticals.

CD	Drug(s)	Ref.
β -CD	Nimesulide, Sulfomethiazole, Lorazepam, Ketoprofen, Griseofulvin, Praziquantel, Chlorthalidone, Etodolac, Piroxicam, Itraconazole, Ibuprofen	56-68
α -CD	Praziquantel	62
γ -CD	Praziquantel, Omeprazole, Digoxin	62, 68, 69
Hydroxypropyl- β -CD	Albendazole, DY-9760e, ETH-615, Levemopamil HCL, Sulfomethiazole, Ketoprofen, Griseofulvin, Itraconazole, Carbamazepine, Zolpidem, Phenytoin, Rofecoxib	70-73, 57, 59, 61, 66, 74-77
Dimethyl- β -CD	Naproxen, Camptothecin,	85, 86
Sulfobutylether- β -CD	DY-9760e, Danazol, Fluasterone, Spiranolactone	71, 80-82
Randomly methylated β -CD	ETH-615, Tacrolimus	72, 83
Randomly acetylated amorphous- β -CD (Ac- β -CD)	Naproxen	84

Stabilization

The drugs must retain sufficient stability not only during storage but also in the gastrointestinal fluids, since reactions which result in a product that is pharmacologically inactive or less active will reduce the therapeutic effectiveness. The main concern in the

pharmaceutical field is, therefore, the rate of deceleration. CDs have been used to stabilize pharmaceutical compounds.^{85, 86}

Thymopentin is a peptide which blocks the stimulation of smooth muscle contractions induced by (+)-anatoxin- α , which is produced by blue green algae. Thymopentin is unstable in aqueous solution and can not be stored in a ready to use form. The activity was retained over 14 months of storage at 25 °C when the drug was complexed with 2-hydroxypropyl- β -CD in aqueous solution. In the absence of the CD, all the activity was lost within 1 week.⁸⁷

Erythropoeten is a glycoprotein hormone which induces an increase in red cell mass. Complexed with 2-hydroxypropyl- β -CD, 100% of the activity was maintained for 10 days compared to only 50% of the activity in the absence of CD. The complexed erythropoeten retained 62% of the activity after 20 days compared to only 24% in the absence of CD.⁸⁸

Nicardipine is light sensitive and decomposes if exposed to light. The rate of photodegradation was reduced by complexing the drug with CDs. CD complexes of nicardipine were irradiated by UV light. Photodegradation was slowed by a factor of 10 with methylated- β -CD, 8 with 2-hydroxypropyl- β -CD, 6.5 with α -CD and 5 with γ -CD.⁸⁹

Digoxin, one of the potent cardiac glycosides, is susceptible to hydrolysis in acidic media. The acid-catalyzed hydrolysis of the glycoside bonds in digoxin is decelerated by the addition of CDs. The hydrolysis of the glycosidic linkage connecting the A-ring of digoxin and the sugar was completely inhibited by β -CD.⁹⁰ When the catalytic hydroxyl groups of the CD are blocked by substituents, their stabilizing effect is enhanced. The β -hydroxyketo-moiety of the E-type prostaglandins is extremely susceptible to dehydration under acidic or

alkaline conditions. Parent CDs showed a positive catalytic effect but the use of methylated β -CD significantly decelerated the degradation reaction. Prostaglandin E_1 has been stabilized by 6-O-(carboxymethyl)-O-ethyl- β -CD in a fatty alcohol propylene glycol ointment, because prostaglandin E_1 is most stable in acidic conditions.⁹¹ Carmofur is extremely susceptible to base and water catalyzed hydrolysis to 5-fluorouracil, which irritates the gastrointestinal tracts. The carmofur can be stabilized by complexing with 6-O-(carboxymethyl)-O- β -CD.⁹² An antitumor drug, O-6-benzylguanine, undergoes hydrolysis under acidic conditions to form guanine and benzyl alcohol. The hydrolysis at pH 4.8 was decelerated by a factor of 220 by complexing the drug with sulfobutyl- β -CD with an average degree of substitution of 4.⁹³ The results of stability enhancement of pharmaceuticals in the presence of CDs are summarized in Table 5.

Absorption Enhancement

The enhanced water solubility of the drug upon complexation with CDs has been found to result in the enhanced bioavailability of the drug.¹⁰³⁻¹⁰⁵ The rate and extent of bioavailability of a poorly water-soluble drug from its CD complex is optimized by adjusting factors affecting the dissociation equilibria of the complex both in the formulation and in the biophase in which the complex is administered. Only a free form of the drug, which is in equilibrium with the complexed one in solution, is capable of penetrating the lipophilic barriers consisting of either mucosal epithelia or stratified cell layers and eventually entering the systemic circulation. Practical formulations usually contain a large quantity of pharmaceutical excipients, which may compete with the drug for the CD cavity. Moreover, the endogenous substances existing at the absorption site may also compete for the CD

Table 5 Effect of cyclodextrins on drug stability.

Effect	Drug	CD	Ref.
Photostability	Promethazine	Hydroxypropyl- β -CD, Dimethyl- β -CD	72
	DY-9760e	Sulfobutylether- β -CD	71
	2-Ethylhexyl-p-dimethyl aminobenzoate	Hydroxypropyl- β -CD	94
Shelf life with unaffected dissolution rates for 4 years	Glibenclamide	β -CD	95
Thermal stability in solid state	Diclofenac sodium	β -CD	96
Stability against intramolecular cyclization in solid state	Quinaril	β -CD, Hydroxypropyl- β -CD	97
Stability to acid hydrolysis and photodecomposition	Doxorubicin	Hydroxypropyl- β -CD, Hydroxypropyl- γ -CD	98
	Acyl ester prodrugs of Ganciclovir	Hydroxypropyl- β -CD	59
Stability against hydrolysis	Digoxin	γ -CD	69
	Rofecoxib	Hydroxypropyl- β -CD	70
	Camptothecin	Randomly dimethylated β -CD	79
	Melphalan	Sulfobutyl- β -CD,	99
	Carmustine	Hydroxypropyl- β -CD	
	Paclitaxel	γ -CD Hydroxypropyl- γ -CD Hydroxypropyl- β -CD	100
Deacetylation or degradation	Spiranolactone	Sulfobutylether- α -CD, Sulfobutylether- β -CD, Hydroxypropyl- β -CD, γ -CD, β -CD	101
Photoreactivity	Flutamide	β -CD	102

cavity. The displacement of the drug from the CD cavity by exogenous and endogenous substances is responsible for acceleration of the drug absorption. Some recent findings on the enhanced drug absorption by hydrophilic CDs administered via different routes are discussed here.¹⁰⁶

Oral Delivery

The commercial viability of the CD-based oral formulations has been established with the marketing of more than 10 products.^{107, 108} Rapidly dissolving complexes of drugs with hydrophilic CDs are best suited for sublingual or buccal administration. This type of drug delivery not only gives a rapid rise in the systemic drug concentration but also avoids intestinal and hepatic first-pass metabolism of the drug. A water soluble complex of digoxin with γ -CD can be formulated into a sublingual tablet to enhance bioavailability and to avoid acid hydrolysis of the drug by gastric juices.¹⁰⁹ Other examples are sublingual administration of tablets containing complexes of steroids with CDs. 2-Hydroxypropyl- β -CD and β -CD polymer supported the absorption of testosterone from the oral cavity and not from the gastrointestinal tracts.^{110, 111}

Rectal Delivery

The release of drugs from suppository bases is one of the important factors in the rectal absorption of the drug. In general, hydrophilic CDs enhance the release of poorly water-soluble drugs from oleaginous suppository bases. In comparison to parent CDs, the methylated CDs are found to significantly enhance the rectal absorption of hydrophobic drugs such as flurbiprofen,¹¹² carmafur¹¹³ and biphenylacetic acid¹¹⁴ from the oleaginous suppository. 2-Hydroxypropyl- β -CD was also found particularly useful for the enhancement of rectal absorption of the above drugs. The most striking effect of 2-hydroxypropyl- β -CD was observed for the rectal absorption enhancement of the anti-inflammatory drug ethyl 4-biphenylacetate, a lipophilic prodrug of biphenylacetic acid.¹¹⁵ There is a great clinical

need for the development of long-active types of rectal preparations for the potent opioid, morphine, in the treatment of intractable chronic pain in advanced cancer patients. Some hydrophilic CDs have been found to enhance the rectal absorption of morphine from the hollow-type oleaginous suppository in rabbits.¹¹⁶

Ocular Delivery

One of the pre-requisite for a new vehicle to be used in ophthalmic preparations is that it is not irritating to the ocular surface, because irritation causes reflex tearing and blinking, which results in a fast washout of the instilled drug. The major problem with eye drops is its inability to sustain high local concentrations of drug. The administration of ophthalmic drugs in gels and in polymer matrixes has been shown to increase the contact time of the drug with cornea, a situation which increases their bioavailability. However, the patient's acceptance of such delivery systems is unsatisfactory. The possible advantages in the use of CDs are increase in solubility and/or stability and avoidance of incompatibilities of drugs, such as irritation and discomfort.¹¹⁷

Hydrophilic CDs, especially 2-hydroxypropyl- β -CD and sulfobutyl- β -CD have been shown to be nontoxic to the eye and are well tolerated in aqueous eye drop formulations. These hydrophilic CDs do not penetrate tight biological barriers such as the eye cornea but enhance the ocular bioavailability of lipophilic drugs by keeping the drugs in solution and increasing their bioavailability at the surface of the corneal barriers.^{118, 119} For example, dexamethasone acetate-2-hydroxypropyl- β -CD complex can be made as an ophthalmic solution. Recent studies have demonstrated that water soluble polymers such as hydroxymethylcellulose and

polypyrrolidone stabilize the complex of dexamethasone with 2-hydroxypropyl- β -CD probably through the formation of a ternary complex, a situation which increases the aqueous solubility in eye drops and enhances the drug penetration into the aqueous humor in humans.¹²⁰ A combination of 2-hydroxypropyl- β -CD with hydroxypropyl-methyl-cellulose has also been shown to improve the topical delivery of carbonic anhydrase inhibitors to the eyes.^{121, 122}

Dermal Delivery

CDs have a significant safety margin in dermal applications and can be used to optimizing the transdermal delivery of drugs intended either for local or systemic use.¹²³ A suitable vehicle must be selected so that CDs fully exert their functions. For instance, the *in vitro* release rate of corticosteroids for water containing ointments (hydrophilic, absorptive or polyacrylic base) is markedly increased by hydrophilic CDs, whereas in other ointments (a fully alcohol propylene glycol or macrogol base) the CDs retard the drug release.¹²⁴ When the hydrophilic ointment containing ethyl 4-biphenylacetate or its CD complexes was applied to the skin of rats, the release of ethyl 4-biphenylacetate from the ointment into the skin was enhanced by heptakis(2,6-di-O-methyl)- β -CD or 2-hydroxypropyl- β -CD, while β -CD had no appreciable effect. In addition, the β -CDs assisted the bioconversion of ethyl 4-biphenylacetate to biphenylacetic acid in the skin and consequently facilitated the delivery of active biphenylacetic acid to subcutaneous tissues, where its action is most desired.^{125, 126}

Alleviation of Local and Systemic Toxicity

The molecular entrapment of a drug into the CD cavity prevents its direct contact with biological surface as well as the entry of the drug into the cells of non-targeted tissues and thus reducing the local irritation.¹²⁷ Pilocarpine was administered as a prodrug, O,O'-dipropionyl(1,4-xylene)bipilocarpine acid diester, with 2-hydroxypropyl- β -CD. The amount of irritation decreased as the amount of CD was increased. At a concentration of 15%, the irritation was reduced to the same level as that with the commercial formulation and the ocular delivery was substantially improved.¹²⁸ A complex of diclofenac was compared to a commercial preparation, Voltaren. Lysis of red blood cells was used to evaluate cellular lysis. The amount of complex needed to lyse the red blood cells was four times higher than for diclofenac alone. The results indicated the suitability of the hydroxypropyl- β -CD to optimize the ophthalmic application of the drug for improved transcorneal permeability and *in vivo* tolerance based upon the haemolysis studies.¹²⁹ CDs also alleviate muscular tissue damage following intramuscular injection of drugs. For example, the intramuscular administration of chlorpromazine in the absence as well as presence of β -CD derivatives to rabbits showed reduced chlorpromazine-induced muscular damage.¹³⁰ CDs diminish the ulcerogenic potency of several acidic antiinflammatory drugs and mask their disgusting smells and tastes when they are administered orally. A similar protection by hydrophilic CDs after rectal and ocular administration of drugs was also described. For example, 2-hydroxypropyl- β -CD significantly reduced the irritation of rectal mucosa in rats caused by biphenylacetic acid both for single and multiple administrations of the complex of ethyl 4-biphenylacetate with 2-hydroxypropyl- β -CD in oleaginous suppositories.¹³¹

Systemic Detoxification

A study has demonstrated that the addition of β -CD to dialysis fluids accelerated the removal of phenobarbital by peritoneal dialysis, thereby proving effective in the treatment of drug overdose.¹³² A retinal-dextran conjugate solubilized by β -CD was reported to be less cytotoxic and retained the ability to inhibit the growth of cancer cells.¹³³ CDs can be used not only as an enabling excipients in pharmaceutical formulations, but also as artificial carriers, for either exogenous or endogenous lipophiles, in the body.¹³⁴ Some natural lipophiles are changed into toxic agents when the organism lacks the ability to transport and redistribute them properly by carrier proteins and their receptor systems. Furthermore, various exogenous lipophiles enter the body and accumulate in fat tissues. Consequently, these exogenous lipophiles may exert toxic action for a very long period. In such cases CDs act as an artificial circulating carrier for the lipophiles in order to redistribute them in extracellular space.

When heptakis(2,6-di-O-methyl)- β -CD was administered parenterally to mice under retinoid induced hypervitaminosis A, the survival rate of poisoned animals was significantly improved.¹³⁵ This preliminary result was the impetus for the use of 2-hydroxypropyl- β -CD to rescue a patient with familial hypervitaminosis A caused by overloading retinal esters in the liver.¹³⁶

Gentamicin, an aminoglycoside antibiotic, is widely used in the clinical treatment of gram-negative infections, but its use is sometimes complicated by the development of drug-induced acute renal failure. CD sulfates, when given intraperitoneally, protected rats against renal impairment due to gentamicin. The protection probably occurs through

interfering with the intracellular events leading from the drug accumulation to nephrotoxicity.¹³⁷

CD Based Delivery Systems

Due to their multifunctional characteristics, CDs have also been found useful in oral drug delivery, peptide and protein delivery, and site-specific delivery. Drug release should be controlled in accordance with the therapeutic purpose and the pharmacological properties of active substances. There has been a growing interest in developing the rate- or time-controlled type oral preparations, because an appropriate drug release from the dosage forms is of critical importance in realizing their therapeutic efficacy. Various CD derivatives have been used for controlled drug release in oral preparations. The hydrophilic and hydrophobic CDs are useful for the immediate release¹³⁸ and prolonged release^{139, 140} type oral formulations, respectively. The delayed release type oral formulation can be obtained by the use of 6-O-(carboxymethyl)-O-ethyl- β -CD.¹⁴¹

Advances in biotechnology have accelerated the economical, large scale production of therapeutically active peptide and protein-based drugs used to combat poorly controlled diseases, making them more readily available for therapeutic use. The progress in molecular biology, however, has not been matched by the progress in the formulation and development of delivery systems for peptide and protein drugs. There are considerable hurdles to be overcome before practical use can be made of therapeutic peptides and proteins because of chemical and enzymatic instability, poor absorption through biological membranes, rapid plasma clearance and immunogenicity. Many attempts have been made to address these

problems by chemical modifications or by coadministration of adjuvants to eliminate undesirable properties of peptides and proteins. CD complexation is an attractive alternative to the above approaches.¹⁴²

Recently, intensive efforts have been made to design systems able to deliver drugs more efficiently to specific organs, tissues, and cells, etc.¹⁴³⁻¹⁴⁵ The CD complexes are in equilibrium with guest and host molecules in water, the degree of the dissociation being dependent on the magnitude of the stability constant. This property of the complex is a desirable quality, because the complex dissociates to free CD and drug at the absorption site, and only the drug in free form enters into systemic circulation. A typical example is the application of 2-hydroxypropyl- β -cyclodextrin to the chemical delivery system developed by Bodor¹⁴⁶ which was used to deliver the polar drugs targeted on the brain.

3. Chiral Recognition by Cyclodextrins

Enantiomerism has always been treated as a rather “special” type of isomerism, probably because the only difference between the enantiomers is the manner in which they interact with polarized light. One enantiomer is therefore rarely regarded as an impurity when present in a sample of the other enantiomer, in contrast to, say, a mixture of two aromatic positional isomers or a mixture of E- and Z-alkenes. In fact, this lack of any difference other than optical activity, only holds in a truly achiral situation and as soon as any chirality is introduced into the environment of a chiral molecule the enantiomers are differentiated. This will clearly be the case when a chiral drug interacts with an enzyme or a receptor, as such biomolecules will typically represent a chiral environment. Furthermore, processes such as

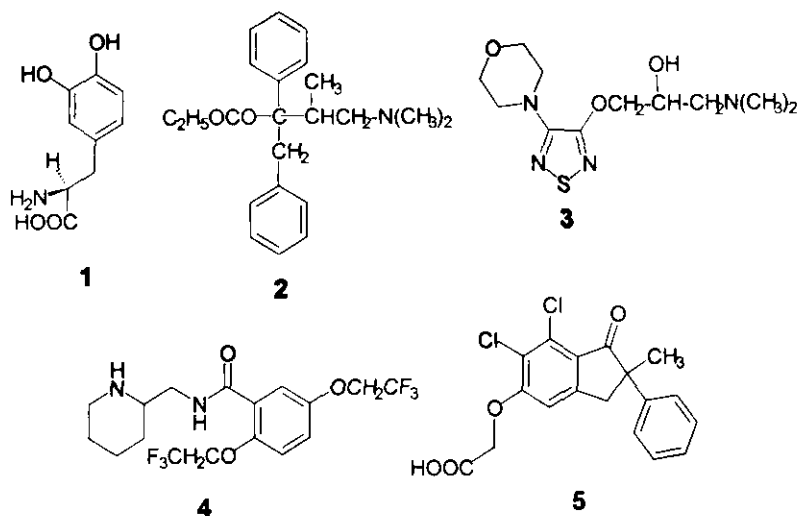
drug absorption, distribution, excretion and metabolism are all potentially subject to enantioselection.

It is important to realize that the other enantiomer of a chiral drug, usually present as a 50% admixture, as the result of chemical synthesis, may be more than just an ineffective version of its mirror image, and may have dramatically different (and potentially undesirable) pharmacological effects in its own right.

L-Dopa (1), α -dextropropoxyphene (2) and S-(-) timolol (3) are commercially available drugs that are marketed as single enantiomer because the antipode produced unwanted side effects. For L-dopa, it was noted during early development that many of the serious side effects, such as granulocytopenia, were due to the D-isomer: the racemate is no longer used in humans.¹⁴⁷ In contrast to flecainide (4) where both enantiomers have been reported to have very similar activity,¹⁴⁸ the isomers of propoxyphene appear to have completely different activities. D-Propoxyphene has analgesic properties whereas its optical isomer L-propoxyphene has antitussive properties but is devoid of analgesic properties.¹⁴⁹

Indacrinone (5) is an interesting drug that has both diuretic and uricosuric activity. Preclinical and clinical studies showed that R-(-)-**5** and its active metabolite are responsible for the diuretic activity, whereas S-(-)-**5** promotes uric acid excretion.¹⁵⁰ Each enantiomer seems to possess very little of the antipode's activity. Similarly, S-(-) enantiomer of **3** was selected for development as a β -blocking agent because the R-(+)-**3** was found useful for the treatment of glaucoma. S-(-)-**3** has been reported to cause fatal β -blockade induced bronchoconstriction when applied topically to treat glaucoma. It becomes obvious from these examples that each enantiomer needs to be evaluated separately before being regulated for use as a drug. The

separation of enantiomers and their characterization are, therefore, of great importance for the pharmaceutical industry.¹⁵¹



Chiral recognition, also often termed as enantiorecognition, refers to the ability of one chiral molecule to recognize the chirality of another molecule. CDs are chiral molecules and exhibit chiral recognition, i.e., they differentiate between enantiomeric species, forming diastereomeric complexes. The magnitude of the enantiorecognition in guest-CD interactions can be characterized by enantioselectivity. Enantioselectivity of recognition is a thermodynamic quantity correlating in some way with the separation factor of enantiomers in separation techniques.

It must be noted that among presently applied chiral selectors only CDs are effectively used in all enantioseparation techniques.¹⁵² In fact, widespread industrial applications of CDs are primarily due to their two properties: complex formation and their chiral recognition ability. A better understanding of the inclusion complex formation and the chiral recognition mechanisms of CDs is, therefore, a subject of great importance.

The basic mechanism of the interactions between CDs and other molecules is known¹⁵³ but there are still questions to be answered. The most critical questions in CD chemistry are: (a) is the inclusion complex formation a prerequisite for chiral recognition by CDs? (b) Does any correlation exist between the binding strength and enantioselective power by CDs? (c) What sites of the CDs are primarily responsible for binding and enantioselective recognition? (d) What are the major forces responsible for binding, and what factors are responsible for chiral recognition.

Of all the techniques used to study chiral recognition, chromatographic techniques and NMR spectroscopy are the most frequently used methods. In chromatography, the separation of enantiomers is usually studied while spectroscopic techniques are used to study differences in other physicochemical parameters of the diastereomeric complexes involving CDs.

NMR spectroscopic studies have provided some of the most detailed information concerning the nature of these interactions and the structure of the diastereomeric complexes involved. The most distinct advantage of the NMR spectroscopy is that it allows the application of racemic samples or non-racemic mixtures of enantiomers for the enantioselective determination of the stoichiometry and equilibrium binding constants of the diastereomeric CD-complexes. Besides, the easier availability of racemic mixtures compared to pure enantiomers offers the possibility of competitive binding studies. This means that the interaction of one of the enantiomer of a racemic mixture with a chiral selector may be studied in the presence of the other enantiomer, which mimics closely the real condition in chiral chromatographic separations.

NMR spectra of enantiomers in an achiral medium are identical because enantiotopic groups display same chemical shifts.¹⁵⁴ Enatiodifferentiation in the spectra require the use of a chiral medium that converts the mixture of enantiomers into a mixture of diastereoisomeric complexes. The simplest application of the chiral discrimination by CDs is an observation of the separation of signals of diastereomeric forms. Such experiments enable a simple estimation of optical purity of the guest molecules and rely on evaluation of relative signal intensities belonging to respective species. Most of the observations of splitting signals due to chiral recognition pertain to ^1H spectra. There are very few examples describing the effect in ^{13}C spectra¹⁵⁵⁻¹⁵⁷ and other nuclei, ^{15}N ,¹⁵⁸ ^{19}F ¹⁵⁹ and ^{31}P .¹⁶⁰

Evidence of chiral recognition is usually the first step for further structural, thermodynamic or theoretical studies. Although the chemical shifts of such species are generally different, the magnitude of the expected effect is not always large enough to be observed. Moreover, it depends on the chemical shift differences between the free and complexed forms of the guest, the actual binding constant and finally on the kinetics of host/guest exchange. Hence, in order to increase the separation of the signals, an excess of CD is usually used and an analysis of the guest spectra is made. In practice, the observation of the separation of ^1H signals due to chiral recognition is easiest and straightforward in the case of relatively strong and narrow signals but may be very difficult for complicated multiplets or broad signals. The observation of other nuclei provides several advantages, which include a larger chemical shift scale and a lack of multiplet structure as in ^1H -coupled spectra.

The spectra of (\pm)-pinene (6) complexes¹⁶¹ with α -CD in D_2O present a typical example of the differentiation in ^1H and ^{13}C signals due to chiral recognition.¹⁵⁷ The corresponding

$\Delta\delta = \delta(+)-\delta(-)$ values are given in Table 6. The ^1H signal separations were observed for almost all protons except H-7 and H-9, whereas, due to their larger chemical shift scale, the $\Delta\delta$ (^{13}C) values were larger by atleast 1 order of magnitude.

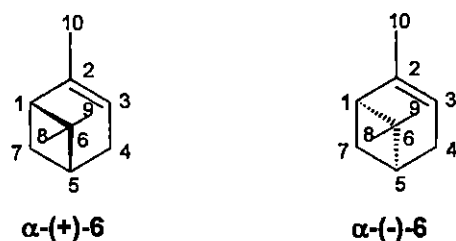


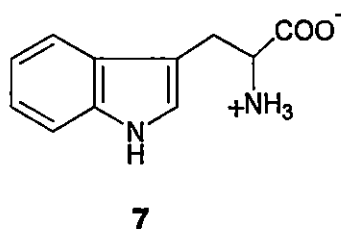
Table 6 Differences between ^1H and ^{13}C complexation induced shifts (CIS) (ppm) of enantiomeric guest signals for the complexes of enantiomeric α -pinene with α -CD in D_2O and DMSO.

Atom	$\Delta\delta$ CIS (D_2O)		$\Delta\delta$ CIS (DMSO)
	^1H	^{13}C	^{13}C
1	-0.04	-0.5	0.01
3	-0.05	-0.2	-0.01
4a	-0.02		
4b	0.00	1.2	0.01
5	0.04	0.3	0.01
7a	-0.02		
7b	0.01	-1.3	-0.01
8	-0.02	-0.3	0.00
9	0.00	-0.4	-0.02
10	0.04	0.2	-0.02

The influence of the magnitude of binding constants on the apparent signal separation is exemplified by the spectra observed for the same system in DMSO solution. In this case, due to greater solubility of free pinene, the binding constant should substantially decrease, consequently the differentiation of the ^1H signals was too small to be observed and the

difference in the magnitude of the ^{13}C chemical shifts were reduced.¹⁶² Thus, as a result the differentiation of the signals of the enantiomeric guest was seen only in the ^{13}C spectrum.^{157, 163}

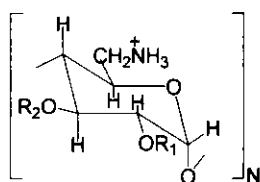
Spin-spin couplings, another NMR parameter, can also exhibit enantiodifferentiation. Since spin-spin coupling cannot be directly read out from the spectra of tightly coupled spin systems, it has seldom been used in chiral recognition studies. Lipkowitz and coworkers¹⁶³ determined complexation induced differences in apparent couplings in the range of 0.01-1 Hz for tryptophan (7) enantiomers complexed by α -CD. The observation of enantiodifferentiation of coupling constants is expected to be more reliable for couplings involving heteronuclei, owing to their usually larger magnitude.



The relaxation rates of nuclei are even more difficult to determine than spin-spin coupling. Special experimental techniques and appropriate data reduction have to be applied in order to obtain their values.¹⁶⁴ On the other hand, relaxation rates of many magnetically active isotopes can provide valuable information on the dynamics of the system under study.^{164, 165}

Few relaxation studies for the complexes of CDs with enantiomeric guests have been reported. Interestingly, not all these works exhibit the differentiation of relaxation rates due to chiral recognition. The observations of differences in the relaxation rates of CD diastereomeric complexes are scarce and all but one is devoted to the studies of longitudinal

relaxation rates. The first systematic study of this effect was carried out for 7-CD complexes in D₂O.¹⁶⁶ Longitudinal relaxation rates for all protons of host and guest molecules were determined in free **7** (0.26-2.3 s⁻¹), free α-CD (1.4-5.0 s⁻¹) and both diastereomeric complexes at a host-to-guest ratio of 1:1. The relaxation rates of **7** increased on complexation but were smaller than those for α-CD protons. These results were interpreted in terms of larger motional freedom of the guest molecules than that of the host. According to authors, larger differential changes in relaxation rates of protons of **7** in the complex with R-enantiomer, as compared to those with the S-enantiomer complex, indicated that the latter enantiomer binds less tightly to α-CD. The cooperative effects of Coulomb interaction and inclusion were assumed to be essential for chiral recognition of anionic phenylacetic acid derivatives by several aminated CDs (**8**, **9**).¹⁶⁷

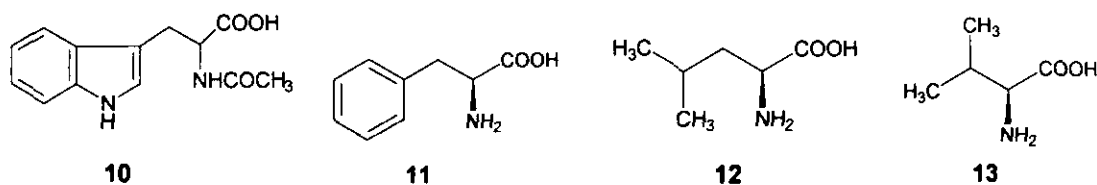


(**8**) N=6, R₁=R₂=H

(**9**) N=7, R₁=R₂=H

Likewise, the importance of electrostatic interactions for chiral discrimination was proved for complexes of charged, cationic and anionic β-CD with various α-aminoacids.¹⁶⁶ It was demonstrated that protonated heptakis(6-amino-6-deoxy)-β-CD favoured the complexation with (S)-enantiomers of N-acetylated tryptophan (**10**), phenylalanine (**11**), leucine (**12**) and valine (**13**) in their anionic form while the native α- and β-CD did not. Enantiodifferentiation was also found for cations of methyl esters of α-aminoacids interacting with the anionic form

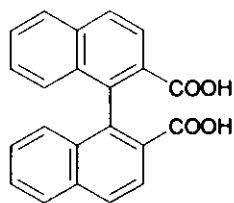
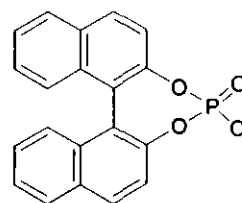
of heptakis(6-(thioglycolic acid)-6-deoxy)- β -CD where the (R)-enantiomers were preferred guests.



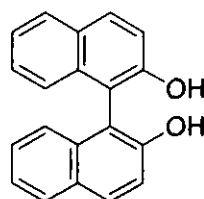
Enantiodifferentiation of anionic tetrahelicene (**14**) by native CDs was studied using binding constants and thermodynamic parameters determined by variable temperature ^1H NMR titration.¹⁵⁷ The binding constant for the (M)-**14** was found to be much higher than that for the corresponding (P)-**14**. However, the 2D ROESY measurements indicated a somewhat deeper insertion of the (P)-form into the CD cavity. The results were attributed to a domination of enthalpy effects in the process of complexation.



The binding constants and enthalpy change (ΔH) and entropy change (ΔS) values were determined for both the enantiomers of binaphthyl derivatives (**15**, **16**) with native and heptakis(2,3,6-tri-O-methyl)- β -CD.¹⁶⁰ The results revealed that the complexation is entropically driven. They also showed that the entropically driven complexation by the permethylated derivative differentiated the enantiomers more effectively.

**15****16**

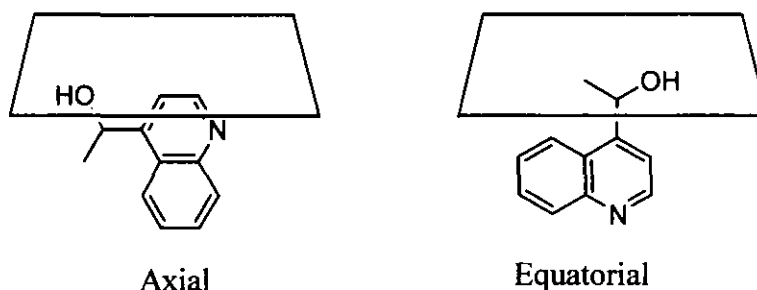
Several studies^{168, 169} reported the stoichiometry of CD complexes of enantiomers in combination with chemical electrophoresis (CE) enantioseparations, but a distinct difference in the stoichiometry of the complexes was observed in only few cases. Thus for example, Kano and coworkers¹⁷⁰ observed different stoichiometries for the complexes of S- and R-1,1'-binaphthyl-2,2'-diol (17) with heptakis(2,3,6-tri-O-methyl)- β -CD. The stoichiometry of S-guest complex was determined to be 2:1 while for R-guest a 1:1 stoichiometry was found.

**17**

An article on the structure determination of CD complexes, using NMR spectroscopy in relation to CE, was published by Yamashoji et al. in 1992.¹⁷¹ Subsequently, several studies were reported^{172, 173} in which the authors tried to explain the quantitative differences observed in the behaviour of chiral selectors based on the structure with guest molecules. All these studies contributed significantly to a better understanding of CD-guest interactions. However, at present it is difficult even with very sophisticated powerful NMR techniques to define the structure and dynamics of the complexes on a level providing the key for the

explanation of sometimes very fine quantitative differences in the enantioselective recognition by different cyclodextrins.

The ^1H NMR titration and ROESY studies of inclusion of 1-(4-quinolyl) ethanol (18) by per-O-methylated- α -CD evidenced the formation of equatorial and axial complexes of (R)- and (S)-enantiomers, respectively.¹⁷⁴ In addition to the determination of the binding constants for both the enantiomers, the free energy changes (ΔG) were also determined.

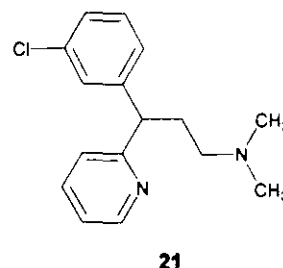
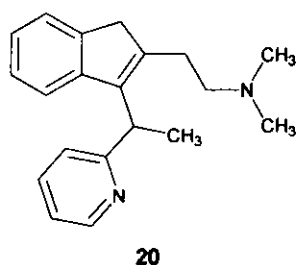
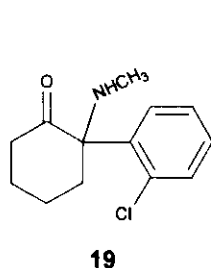


18-(per-O-methylated)- α -CD complexes

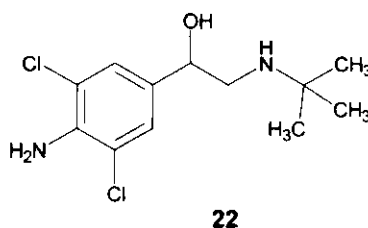
The screening of the affinity patterns of a wide range of chiral guests towards CD-type chiral selectors using CE revealed that the affinity pattern may change depending on the type and position of the substituents on the CD rim and on the CD cavity size.¹⁷⁵ These studies help in elucidation of possible structural mechanism of qualitatively different behavior of the CD type chiral selectors in CE, in particular, for examples in which the affinity of the enantiomers is opposite towards given chiral selectors.

The enantiomers of the anesthetic drug ketamine (19) possesses an opposite affinity pattern towards native α - and β -CD.¹⁷⁶ ROESY spectroscopy indicated that there are no dramatic differences in the structures of the corresponding intermolecular complexes. The enantiomers are just more deeply included in the cavity of β -CD compared to α -CD.¹⁷⁷ Similar to this, no

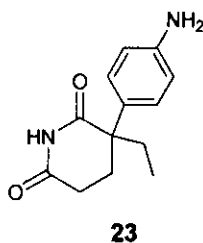
clear differences besides the extent of intermolecular inclusion could be observed between the structures of complexes of dimethinden (20) and chlorpheniramine (21) with β -CD and heptakis(2,3,6-tri-O-methyl)- β -CD although the enantiomer affinity patterns were opposite for all three analytes towards these two CDs.^{177, 178}



In other examples, however, significant differences in the structures of intermolecular complexes could be observed. The enantiomers of clenbuterol (22) showed opposite affinity toward native β -CD and heptakis(2,3-O-diacetyl)- β -CD in CE experiments. The splitting of the resonance signals due to complexation induced chemical shifts of the protons of the enantiomers was primarily observed for the aromatic protons in the case of β -CD and for the protons of the tert-butyl moiety of **22** in the case of heptakis(2,3-O-diacetyl)- β -CD. These data indicate that the aromatic part of **22** is mainly involved in the interaction with β -CD while tert-butyl moiety is involved in the interaction with heptakis(2,3-O-diacetyl)- β -CD.¹⁷⁸

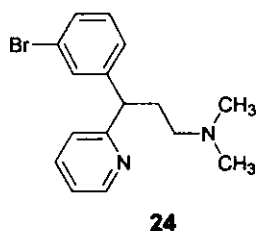


The chiral cholinergic drug aminoglutethimide (**23**) shows opposite affinities in CE towards β - and γ -CD.¹⁷⁹ The detailed NMR spectroscopic study confirmed the deep inclusion of the p-aminophenyl moiety of **23** into the β -CD cavity entering from the wider secondary side while the p-aminophenyl ring enters the γ -CD cavity from the narrower primary side. The glutarimide ring is apparently less involved in the complex formation. However, the involvement of the methyl group in complex formation cannot be completely ruled out.



The complexation of antihistamine drug brompheniramine (**24**) with β -CD and heptakis(2,3,6-tri-O-methyl)- β -CD was studied by 1D-ROESY experiments in solution.¹⁸⁰ For the complexes of (+)-**24** with both CDs unambiguous confirmation was obtained indicating the inclusion of the 4-bromophenyl moiety of the drug into the CD cavity. In addition, in the case of (+)-**24** complex with β -CD, the inclusion of the maleate counter ion into the β -CD cavity was also indicated, but this contradicts simple geometric considerations and the assumption that the stoichiometry of the complex is 1:1. The contradiction was solved by the X-ray crystallographic study performed on the monocrystals obtained from a 1:1 aqueous solution of (+)-**24** maleate and β -CD. The stoichiometry of the complex was found to be not 1:1 but 1:2 and the brompheniramine maleate is sandwiched between two molecules of β -CD. The 4-bromophenyl moiety of the drug enters the cavity of one of the

β -CD molecules whereas the cavity of another β -CD molecule is occupied by the maleate counter anion.



In a recent study, 27 cationic chiral analytes were resolved by CE using native β -CD and heptakis(2-O-methyl-3,6-di-O-sulfato)- β -CD, having both primary and secondary CD rims with 14 bulky sulfate substituents. The bulky substituents on both sides of the cavity entrance may hinder inclusion complex formation between chiral analytes and heptakis(2-O-methyl-3,6-di-O-sulfato)- β -CD. For 12 of 16 chiral analytes resolved with both chiral selectors the enantiomer migration order was opposite. Analysis of the structures of analyte-CD complexes in solution indicated that, in contrast to mainly inclusion type complexation between chiral analytes and β -CD, external complexes were formed between the chiral analytes and heptakis(2-O-methyl-3,6-di-O-sulfato)- β -CD.¹⁷²

The enantiomers of **23** enantioselectively bind to heptakis(2-O-methyl-3,6-di-O-sulfato)- β -CD and are resolved with this chiral selector in CE. In addition, significant complexation induced chemical shift differences were observed for the protons of **23** enantiomers in NMR spectrum of the complex. It was established by 1D ROESY experiments that **23** most likely does not form an inclusion complex with heptakis(2-O-methyl-3,6-di-O-sulfato)- β -CD. Thus, inclusion complex formation between CDs and their chiral guests does not seem to be a necessary prerequisite for chiral recognition.

In conclusion, multiple forces involved in guest-CD interactions make understanding of guest binding and chiral recognition mechanism by CDs extremely difficult. Molecular modeling studies when used in combination with instrumental techniques, especially with ROESY experiments in NMR spectroscopy and X-ray crystallography may significantly contribute to the understanding of the nature of the intermolecular forces responsible for guest-CD interactions and chiral recognition.

4. Forces Involved in Cyclodextrin Complexation

The understanding of the driving forces in the inclusion complexation of CDs is fundamentally important not only for CD chemistry but also for supramolecular chemistry as a whole. A large number of studies have been carried out on the subject and have been reviewed earlier.¹⁸¹ Nevertheless, it is often claimed that the driving forces leading to CD complexation still remain a controversial subject. Here we will only discuss the interactions between the substrates and the cavity wall of the CD.

Electrostatic Interactions

Electrostatic interactions include all kinds of electrostatic forces between permanent charges, dipoles and higher dipoles, present in the system. Three types of electrostatic interactions are usually most important namely ion-ion, ion-dipole and dipole-dipole interactions. As native CDs are neutral molecules, the ion-ion interaction does not occur in CD complexation, unless the CD is appropriately substituted.¹⁸²

The ion-dipole interaction, on the other hand, is expected to take place in CD complexation since CDs are polar molecules though the occurrence of this interaction is difficult to establish. The ion-dipole interaction should increase with increasing ionic charge of the guest. It can be expected that dianions such as SO_4^{2-} and CO_3^{2-} will bind more tightly with CDs than ions such as ClO_4^- and NO_3^- . However, though the complexation of CD with ClO_4^- and NO_3^- has been observed experimentally, no complexation could be detected for SO_4^{2-} or CO_3^{2-} .¹⁸³ The ion-dipole interaction, in aqueous solution, is not necessarily favourable since interaction between substrate and water is also strong. Chujo et. al.¹⁸⁴ first calculated the dipole moments of CDs, from the published X-ray crystal structures, in the range of 10-20 D suggesting that CDs are highly polarized. Later studies showed that dipole moments of CDs are highly susceptible to the chemical environment.¹⁸⁵ Smaller dipole moments in the range of 2-4 D were obtained for the CDs optimized by various theoretical methods. Since CDs have modestly large dipole moments, this must play an important role in their complexation.

Dipole-Dipole Interaction

Model studies for the complexation of α -CD with several substituted benzenes such as benzoic acid, p-hydroxybenzoic acid and p-nitrophenol^{186, 187} were carried out and it was found that dipoles of the guest are antiparallel to that of the host. Interestingly, as the magnitude of the guest dipole increases, so does the value of the CD dipole but in opposite direction. Thus it was concluded that dipole-dipole interaction plays an important role in stabilizing the complex as well as determining its orientation.

The free energy relationship analyses also prove the importance of dipole-dipole interaction in CD complexation. The correlation studies between the binding constants of α -CD with

4-substituted benzoic acids and the Hammett σ constants of the substituents were carried out.¹⁸⁸ The results showed that as the $-\text{COOH}$ group always stays at the positive end of the dipole of the host, it is readily understandable that binding is enhanced by electron release from the para substituents. However, in the case of complexation of α -CD with benzoate anions, it is the electron withdrawing para substituent that favours the binding.¹⁸⁹ This is again caused by the dipole-dipole interaction, because in the anion complexes, the carboxylate group stays at the negative end of the dipole of the CD. Davies et. al.¹⁸⁹ also pointed out the importance of the dipole-dipole interaction in CD complexation based on the correlation studies. The Hammett σ values were used to reflect the electronic effects of the substituents in the 1, 4-disubstituted benzenes. It was observed that for neutral 1, 4-disubstituted benzenes, the group with a larger σ value is bound in the narrower end of the CD cavity because of the favourable dipole-dipole interaction energy. The conclusion has been successfully applied to a number of systems¹⁹⁰ and it was found that the exceptions to the rule such as complexation of α -CD with para substituted aromatic sulfides, sulfoxides, sulfones and ketones are caused by steric hinderance.¹⁹¹

Hamai et. al.¹⁹² studied the effect of CD complexation on the acidities of several phenol derivatives such as 4-nitrophenol, 4-cyanophenol, 4-bromophenol and 4-methoxyphenol which also supported the above results. It was found that, except for 4-methoxyphenol, the acidities of phenols were enhanced as a result of CD complexation. The behaviour was thought to be due to the dipole moments of the phenols, which are usually directed from the hydroxyl group to the para substituent. Thus, the dipole-dipole interaction was concluded to be an important factor in CD complexation.

Yasuda et. al.¹⁹³ recently performed scanning tunneling microscopy studies on self-assembled α -CD inclusion complexes with water, methanol and 4-nitrophenol. The observed structures of α -CD-water and α -CD-methanol complexes were different from that of α -CD-4-nitrophenol complex. It is believed that the difference reflects the important role of the dipole-dipole interaction in CD complexation.

van der Waals Interactions

The presence of van der Waals forces, which seems to mean either the induction and dispersion forces combined or the dispersion force alone, in CD complexation is reasonable. The involvement of van der Waals interaction in the CD complexation has been claimed by many workers. It is generally believed that the hydrophobic interaction between two non-polar molecules is with a positive enthalpy, the observation of a negative enthalpy change in CD complexation is often considered to indicate the dominance of van der Waals interaction instead of hydrophobic interactions.¹⁹⁴ However, as CD complexation is a complicated process, the above argument is not always correct.

The correlation analysis between the binding strength and structural feature of the substrate is a reasonable method to show the involvement of van der Waals interaction in CD complexation. For instance, both the induction and dispersion forces depend on polarizability, which in turn is related to molecular size and electron density, and so to the correlation variables like molar refraction, molecular volume, surface area, molecular weight etc. Thus the correlation between the strength of binding and the above parameters is at least indicative of the importance of van der Waals interaction in CD complexation.¹⁹⁵

The involvement of van der Waals interaction in CD complexation can also be shown by the structure of the complexes. In fact, numerous studies have revealed that bulky guest molecules are in close van der Waals contact with the CD cavities.¹⁹⁶ Interestingly, sometimes van der Waals interaction might be so strong that the hydrophobic but bulky side of the guest molecule can enter the CD cavity. Moreover, the fact that CDs can form stable complexes with the guest molecules in pure organic solvents such as DMF, DMSO and even heptanes evidently demonstrates that van der Waals interaction is essentially important.^{197, 198}

The involvement of van der Waals interaction in CD complexation has also been shown by molecular modeling¹⁹⁹ which is usually performed with molecular mechanic and molecular dynamic calculations. Most of the modeling studies were performed in the gas phase so that the solvent effect plays no role in the results. Many authors concluded, from these calculations, that van der Waals interaction makes the major contribution to the formation of CD complexes. This conclusion is not unexpected because in the calculations the energetic contributions from the dehydration and hydration of the host, guest and their complex, and from the reorganization of the solvent molecules were not taken into consideration. Whether, or not, van der Waals interaction plays a major role in CD complexation in solution is not clear.

It should be mentioned that van der Waals interactions also exist between the solvent molecules and the substrates of CD. Thus, in the CD complexation the substrate is exchanging one set of van der Waals interaction (with the solvent molecules) for another set (with the CD cavity). In fact, this type of exchange is the reason why the ion-dipole interaction is not significant in CD complexation. However, as the polarizability of water is

much lower than that of the organic substrate lining the CD cavity, it is expected that van der Waals interaction should be stronger between CD and the substrates than between water and the substrates. As a result, van der Waals interaction has a positive contribution to complex stability. This effect can be shown by the complexation of CDs with inorganic ions such as ClO_4^- and NO_3^- .²⁰⁰ Apparently, hydrophobic interaction cannot make a contribution in these systems. As the ion-dipole interaction might be stronger between water and the ions than between CD and the ions, the only possible driving force leading to complexation is van der Waals interaction.

Hydrophobic Interaction

The role of hydrophobic interaction in CD complexation is a controversial problem. Traditionally, hydrophobicity was considered to be the result of the enhanced structure of the water molecules in the near vicinity of the non-polar solute, which would bring about a usually large entropy loss during the hydration.²⁰¹ However, neither the neutron scattering measurements nor the computer simulations indicated any evidence that the structure of the water of hydration close to a non-polar solute was more ordered than that of water in the bulk.²⁰²

In the experimental studies, however, the association of non-polar molecules in water is usually found to be with positive enthalpy and positive entropy change. This has long been taken as the experimental signature of hydrophobic interaction. The fact that most of the experimental enthalpy and entropy changes of CD complexation are negative seems to indicate that the hydrophobic interaction is not an important driving force in CD molecular recognition/complexation. It was suspected that the above experimental observation was not

representative enough, possibly, because all the guest molecules that had been studied were not sufficiently hydrophobic. Thus, the α -CD complexation with 1-adamantanecarboxylate was studied, and the observed positive entropy was believed to settle the issue.²⁰³ Unfortunately, a reinvestigation of the system showed that the entropy change is still negative.²⁰⁴

The above problem can be settled if we notice that in CD complexation many interactions other than the hydrophobic interaction are also involved. For example, unlike that in the aggregation of two small non-polar molecules, the van der Waals interaction between CD and the guest is quite strong. As the interaction is attractive in nature and it tends to restrict the conformational freedom of the complex, it is possible that the total enthalpy and entropy of the complexation are both negative in spite of the presence of the hydrophobic interaction. Moreover, it is well known that the transfer of non-polar gases into water is associated with a large positive heat capacity change. Therefore, the fact that CD complexation is often accompanied with a large negative heat capacity change also demonstrates that the hydrophobic interaction is important in association.²⁰⁵

In addition to using the thermodynamic criteria, there are several other methods to show the involvement of hydrophobic interactions. In CD chemistry, the most compelling evidence in favour of the presence of hydrophobic interaction is the repeated observation that in the CD complexes the most non-polar portions of the guest molecules are usually enclosed in CD cavities.

The involvement of the hydrophobic interaction in CD complexation can also be shown by the correlation analyses as, in general, increasing the hydrophobicity of the substituents of

the guest enhances the complexation.²⁰⁶ Parameters of hydrophobicity including the partition coefficient $\log P$ and the hydrophobic surface area are frequently chosen. Sometimes, the correlation between the binding strength and the number of the carbon atoms of a homologous series of substrates is also taken as evidence of hydrophobic interaction. As an increment of ~ 3.0 KJ/mol in the standard free energy of complexation for each methylene group is observed, which is close to the value in the transfer of homologous organic compounds from water to hydrocarbon solvent, it is repeatedly suggested that the binding mechanism of CD is of a hydrophobic nature.²⁰⁷

Another evidence of the hydrophobic interaction is that the strength of the CD complexation is usually weakened upon the addition of organic co-solvent.²⁰⁸ Addition of inorganic salts, on the other hand, tends to strengthen the binding, simply because it makes the bulk solution more polar. The binding constants of CD complexes also increase when D_2O is used as the solvent instead of H_2O which might be caused by the fact that hydrophobic interaction is stronger in D_2O than in H_2O .²⁰⁹

Hydrogen Bonding

The role of the hydrogen bonding in CD complexation has been well established for the complexes in solid state.²¹⁰ The study of a number of crystal structures of CD complexes have clearly shown the well defined hydrogen bonding between the substrates and the hydroxyls of CDs. Usually, the host-guest hydrogen bonding is restricted to the primary O(6)-H groups of CDs because they are flexible and can rotate, in contrast to the secondary O(2)-H and O(3)-H groups which are rigid due to the preferred 4C_1 form of the glucose units.

However, it should be mentioned that sometimes there are also C-H...O²¹¹, C-H...N²¹² and C-H... π ²¹³ interactions between the cavity wall of CDs and the guest molecules.

On the other hand, the role of the hydrogen bonding in CD complexation in aqueous solution is still controversial. The primary reason for the problem is that water can compete with CDs to form the hydrogen bonds with the substrate molecules. For example, molecular dynamic calculations on the complexation of α -CD with p-chlorophenol and p-hydroxybenzoic acid in water clearly indicated that the hydrogen bond is rarely formed between CD and the substrates.²¹⁴ Thus, it was concluded that hydrogen bonding plays a minor role in complexation. Besides, it has been demonstrated that although in the solid complex of α -CD with 4-fluorophenol the OH group of phenol is hidden inside the CD cavity,²¹⁵ in aqueous solution the F atom remains inside and OH group outside the CD cavity.²¹⁶

Nevertheless, examples of hydrogen bonding in CD complexation in aqueous solution have been shown by some authors. For instance, in the study of the complexation of γ -CD with pamoic acid, the large observed binding constants were thought to indicate the occurrence of hydrogen bonding between the carboxylate of the guest and a secondary OH of CD.²¹⁷ Sometimes, the occurrence of hydrogen bonding in CD complexation can be detected with spectroscopic methods. For example, Takahashi et. al.²¹⁸ used the ¹H and ¹⁵N NMR techniques to study the interaction of aspartame with β -CD in aqueous solution. It was concluded that the amide part of aspartame was hydrogen bonded in CD complexation in aqueous solution. Recently, Chen et. al.²¹⁹ studied the pH dependance of the complexation of 3-hydroxynaphthalene-2-carboxylic acid with β -CD. It was found that with increasing pH (pH<11), the binding constant decreases probably because the deprotonated substrate is more

hydrophilic. However, at $pH > 11$ the binding constant increases as the pH value rises. The behaviour was thought to be due to the hydrogen bonding between the deprotonated secondary OH of CD and the hydroxyl group of the guest at the pH range. After β -CD is permethylated, there is a little change in the binding constant at $pH > 11$ with increasing pH value, presumably because permethylated β -CD cannot be deprotonated under the same conditions. Thus it was concluded that hydrogen bonding plays an important role in the CD complexation.

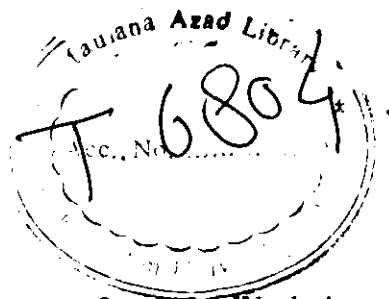
Relief of Conformational Strain

The conformation of a CD in the solid state is usually less symmetrical than that in solution. The crystalline packing and the presence of water molecules in the solid state are, probably, responsible for this behaviour. Saenger and coworkers²²⁰ assumed that the deviation from the symmetrical conformation of the CDs in the solid state constitutes a store of energy whose relief upon complexation is a driving force for the complexation. This was identified as an “induced fit” mechanism. However, β - and γ -CDs exist in nearly symmetrical conformations in solid state, yet their complexes tend to be stronger than those of α -CD rendering the doubts about the strain relief hypothesis. Actually, the hypothesis is not relevant to the complexation of CDs in solution. Though it is possibly true that a CD in the solid state has a higher conformational energy than that in solution, the thermodynamics of the CD complexation in solution does not involve the energy of a solid state CD. Eftink et. al.²²¹ pointed out that conversion of the α -CD from its distorted to symmetrical conformation must cost energy, the process cannot be a source of energy for the complexation. Thus there seems no support for the relief of conformational strain as a driving force for the CD complexation.

Release of Cavity-Bound High-Energy Water

Two water molecules are present in the α -CD cavity in the solid state. As the CD cavity is relatively non-polar, the water molecules included in the CD cavity should lack the complement of stabilizing hydrogen bonds that would be available to them in the bulk aqueous solution. Thus the water molecules in the CD cavity are energy-rich than those in the bulk solution whose release upon complexation was postulated as a driving force leading to the complex formation.²²² This hypothesis was largely developed around the observations on α -CD.

Takagi et. al.²²³ have tried to estimate the thermodynamics of inclusion separately from solvation effect which supports this hypothesis but the problem with the high energy water hypothesis is that it focuses on the water and neglects the CD or more generally it fails to consider the energetics of the entire system. The cavity bound water molecules may well be enthalpy rich but they should have more conformational freedom than the water molecules in the bulk solution because of the lack of hydrogen bonding. Although, the release of the cavity-bound water is accompanied with a negative enthalpy change, the free energy change of the process is not necessarily negative. The reorganization of the solvent molecules is actually a process of enthalpy-entropy compensation without any free energy contribution. As a result, the release of cavity-bound high-energy water is not considered a driving force for the complexation.



Charge-Transfer Interaction

Charge-transfer interaction is usually considered a type of van der Waals interaction.²²⁴ It needs to be discussed separately, however, since in the area of CD chemistry the term van der Waals interaction usually refers to the combination of induction and dispersion forces. In the CD chemistry, in addition to the charge-transfer interaction between the substituent groups of CDs and the guest,²²⁵ charge-transfer interaction between the CD skeleton and the substrate has also been observed and it has been recently suggested to be a driving force in CD complexation. The role of charge-transfer interaction in the CD complexation can be shown by the fact that (1) the binding constant of α -CD complex of the 1, 4-dicyanobenzene radical anion is 45 times larger than that of neutral 1, 4-dicyanobenzene- α -CD complex²²⁶ (2) the binding constant of the β -CD complex of neutral 10-methylphenothiazine is 35 times smaller than that of the 10-methylphenothiazine radical cation complex²²⁷ (3) the binding constant of α -CD with the singlet xanthone is much more stable than that with the triplet one.²²⁸

Conclusion

The driving forces leading to the inclusion complexation of CDs should include the electrostatic interaction, van der Waals interaction, hydrophobic interaction, hydrogen bonding, and the charge transfer interaction. However, enthalpy-entropy compensation, release of conformational strain and release of cavity-bound high-energy water are not energetically contributive to the complex formation. Furthermore, van der Waals interaction and hydrophobic interaction constitute the major driving forces for CD complexation whereas electrostatic interaction and hydrogen bonding can significantly affect the conformation of a particular inclusion complex.

General Discussion

NMR spectroscopy has become the most important tool for the structural elucidation of organic compounds, particularly in the solution state. The method is of increasing significance for most CD application studies. There are few alternatives to NMR spectroscopy in the CD related studies.²²⁹⁻²³²

As with many carbohydrates, it is often difficult to obtain single crystals of CD derivatives and then to analyze them by X-ray crystallography, or by neutron diffraction. Other techniques such as fluorescence, UV/visible spectroscopy, calorimetry etc. play a major role in measuring complexation energetics with CDs but usually provide very indirect and qualitative information about inclusion modes and geometries.

Structure determination is of particular importance for supramolecular host-guest complexes, which are the basis of most CD applications in medicine, catalysis, separation and sensor technology and also food chemistry. Pharmaceutical uses of CDs for drug protection or targeting now legally require structure determination of the administered compounds. NMR spectroscopy is also becoming an important tool for *in vitro*, in future perhaps even for *in vivo*, studies of CD interactions with biological macromolecules such as nucleic acids, proteins, or cell membranes. The most obvious incentive to use NMR techniques for the investigation of CD complexes is the interest to understand the driving forces and binding modes in these non-covalent associations, and then to make optimal use of these factors for new applications. It should be remembered that the driving force for CD inclusion often is of solvophobic nature and that most CD applications involve action in a liquid matrix, which emphasizes again the role of NMR spectroscopy as the most important method applicable in solution.

After the first publication on the use of NMR spectroscopy to study inclusion phenomenon by Demarco and Thakkar,²³³ there has been a virtual explosion of such studies. The older work was restricted to the observation of few CD protons, mostly at the anomeric centers which were sufficiently separated from other strongly coupled signals. The advent of high-field instruments and in particular 2D methods has completely changed the situation, and the possibilities of now available NMR techniques are far from being exploited. Nuclear Overhauser Effects (NOEs) have already become a major tool in structural studies of complex biomolecules.

The spectacular advances of NMR techniques, have led to much more detailed structural information of CDs and their complexes. These tasks represent a fascinating challenge for the NMR spectroscopist in view of the high complexity of the underlying cycloamylose ¹H NMR spin systems.²³⁴⁻²³⁶ These are characterized by signals which, apart from the anomeric proton, absorb in a range of only 0.5 ppm and are strongly coupled. In addition, the shielding effects of the CD cavity on the included guest molecules are limited to a few tenths of a ppm at most, as a consequence of a host framework being built up entirely of single, less polar and polarizable bonds and thus weak shift tensors.

The aim of the present discussion is not to discuss or even to mention all the work on the study of the CD complexes using NMR spectroscopy. Instead we will illustrate the use of NMR techniques in the structure elucidation of CD inclusion complexes with some representative examples, yet without a special focus on this method.

Demarco and Thakkar²³³ first noticed the highfield chemical shift changes in the β -CD cavity protons, namely H-3' and H-5', in the presence of a variety of aromatic substrates in aqueous solution and envisaged from these observations that the aromatic ring is positioned in the β -CD cavity. This observation later became the basis for NMR spectroscopic study of the CD inclusion complexes. He used 100 MHz instrument for these studies which is actually not suited for this type of work because all the CD protons, except H-1', resonate in the 0.5 ppm range and are not easily distinguished. He established the assignment for each proton on the basis of analysis of individual splitting patterns and coupling constants at 220 MHz, decoupling experiments and expected chemical behaviour i.e. H-3' and H-5', being 1, 3-diaxial to the C₁ axial oxygen should resonate at lower fields than the H-2' and H-4' protons.

At magnetic fields above 9.4 T, corresponding to 400 MHz for the ¹H NMR spectra, the dispersion is high enough to locate, in conventional one dimensional spectra, most of the CD protons, eased by the high symmetry of the macrocycles. The ¹H NMR spectrum of β -CD in aqueous solution has already been assigned in detail. It has been established that, on the NMR time scale, all the seven glucose units have identical conformations and the molecule is highly symmetrical. Furthermore, the magnitude of the vicinal coupling constants J_{1,2} through to J_{4,5} are consistent with the C₁ chair form for the glucose units of CDs.²³⁷

It is now well accepted that the signals of the cavity protons move highfield when the guest molecule or a part of it enters the CD cavity. The chemical shift change data

obtained from ^1H NMR titration experiments can be used to determine stoichiometry, binding constant and is thus helpful in the determination of structure of the complex.

The magnitude of the chemical shift changes for the protons positioned in the CD-cavity, $\Delta\delta_{\text{H-3'}}$ and $\Delta\delta_{\text{H-5'}}$, increases with an increase in the concentration of the guest²³⁸ while that for guest protons increases with an increase in the concentration of the CD.²¹⁷ The magnitude of the $\Delta\delta_{\text{H-3'}}$ and $\Delta\delta_{\text{H-5'}}$ also depends on the nature of the guest. The chemical shift changes are high in the case of aromatic guests while these are relatively small if the aliphatic guest enters the CD cavity. Salbutamol (25) forms a 1:1 inclusion complex with β -CD whose structure has been confirmed by detailed NMR spectroscopic and molecular modeling studies (Fig. 4).²³⁹ It has been established that aliphatic part of the guest enters the CD cavity. Fig. 5 shows very small shift changes in the β -CD cavity protons in the presence of salbutamol. On the other hand these shift changes are quite large when aromatic ring of (1S, 2R)-(+)-ephedrine (26) enters the β -CD cavity.²⁴⁰

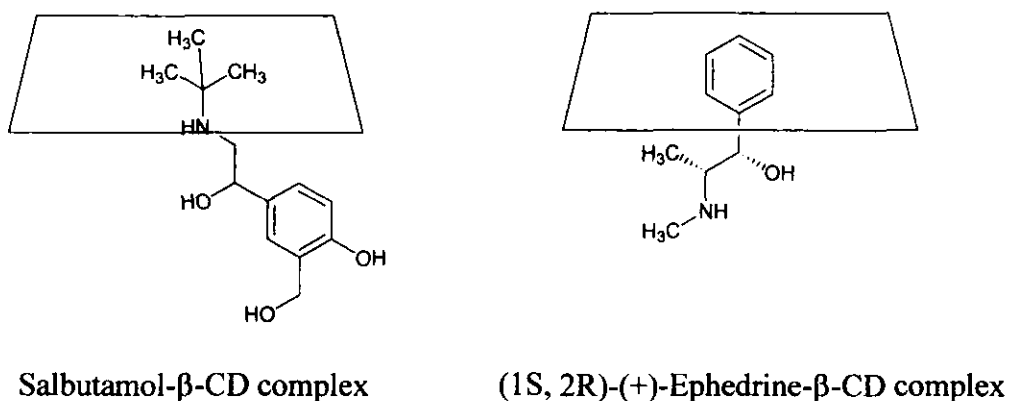


Fig. 4 Structures of inclusion complexes of salbutamol and (1S, 2R)-(+)-ephedrine.

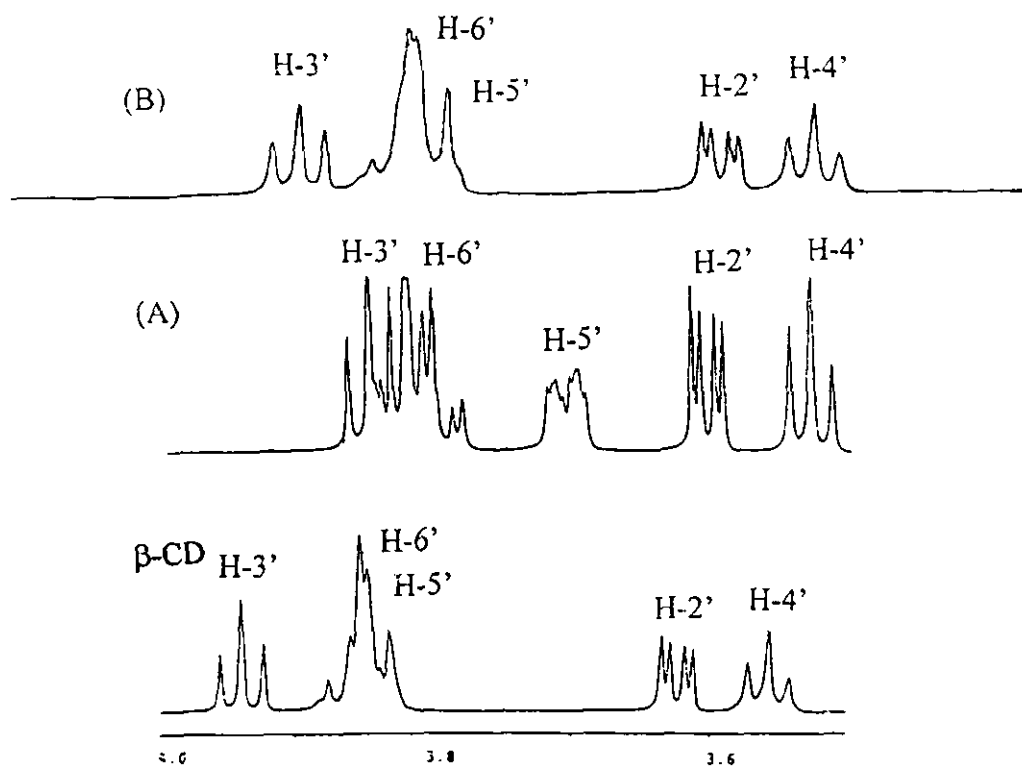


Fig. 5 Comparative chemical shift changes in the β -CD cavity protons upon inclusion of an aromatic guest (A) and an aliphatic guest (B). The $[H]/[G]$ is same in both the cases.

The CDs generally act as one site ligand and the guest enters the cavity from wider rim side. This is always true for α -CD but in the case of β - and γ -CDs, complexes formed by the penetration of the guest from the narrower rim side have also been reported.²⁴¹ The relative chemical shift change data for the cavity protons ($\Delta\delta_{H-3'}$ and $\Delta\delta_{H-5'}$) is sometimes taken as an evidence for the mode of penetration of the guest. It has been suggested that in the cases where $\Delta\delta_{H-3'} > \Delta\delta_{H-5'}$ the guest entry is from wider side¹⁶⁰ and vice versa.²⁴² This statement may be true only when the guest is bulky but can not be generalized.

Kano¹⁶⁰ studied the complexation of pure enantiomers of binaphthyl derivatives with various CDs. The chemical shift changes observed for H-3' of heptakis(2, 3, 6-tri-O-methyl)- β -CD (TMe- β -CD) were quite high compared to those for H-5' ($\Delta\delta_{\text{H-3}'} > \Delta\delta_{\text{H-5}'}$) in the presence of 1, 1'-binaphthyl-2, 2'-diyl hydrogen phosphate (27). It was established that **27** is shallowly bound to the wider side of the TMe- β -CD cavity. The guest being bulky cannot penetrate deep into the cavity. Moreover, the mode of penetration of the two enantiomers was found different. (Fig. 6)

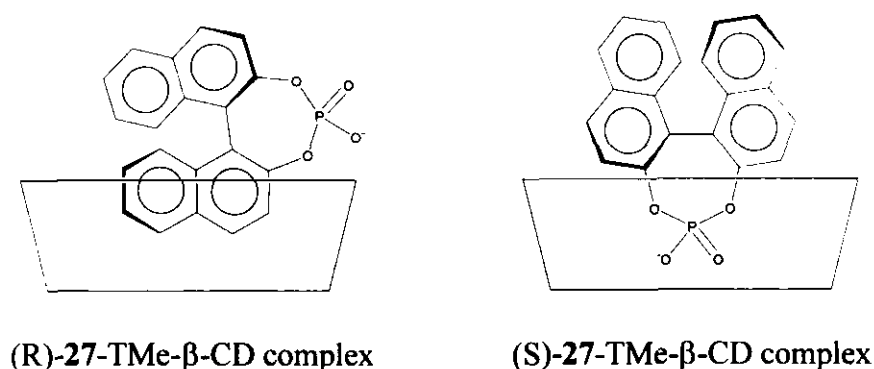
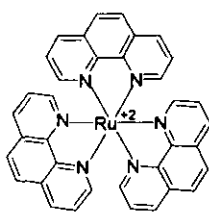
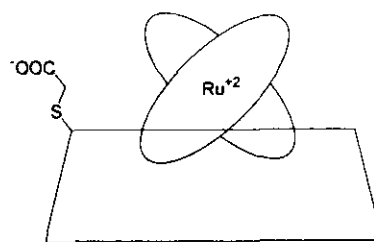


Fig. 6 Modes of penetration of two enantiomers of **27** into the β -CD cavity.

He also studied the complexation of several helical metal complexes with modified CDs. Λ -Ru(phen)₃⁺² ion (**28**) forms a 1:1 complex with heptakis(6-carboxymethylthio-6-deoxy)- β -CD (per-CO₂⁻- β -CD) (Fig.7). It has been shown that the guest approaches the cavity from narrower rim side and for this complex the chemical shift for H-5' was quite high compared to that for H-3' ($\Delta\delta_{\text{H-5}'} > \Delta\delta_{\text{H-3}'}$).²⁴² These examples show that the relative values for $\Delta\delta_{\text{H-3}'}$ and $\Delta\delta_{\text{H-5}'}$ can be used in support of the mode of penetration of guest into the CD cavity.²⁴³

**28**Model of **28**-(per-CO₂⁻)-β-CD complex**Fig. 7** Mode of inclusion of **28** into the (per-CO₂⁻)-β-CD cavity.

Rekharsky,²⁴⁰ however, made a detailed ¹H NMR study of the CD inclusion complexes of a variety of guests and showed that while the magnitude of the chemical shift changes for H-3' and H-5' protons is a quantitative measure of the stability of the complexes, their ratios, $\Delta\delta_{H-5'}/\Delta\delta_{H-3'}$, are related to the depth of penetration of the guest into the CD cavity. In all the studied cases, the guest approached the β-CD cavity from wider rim side though $\Delta\delta_{H-5'} > \Delta\delta_{H-3'}$. He showed that higher magnitude of $\Delta\delta_{H-3'}$ and $\Delta\delta_{H-5'}$ values is due to higher stability of the complex. On the other hand, their ratios, $\Delta\delta_{H-5'}/\Delta\delta_{H-3'}$, which were found as high as 1.2-3.0 for β-CD, indicate a deep penetration of the guest into the host cavity. It becomes obvious from these examples that the use of relative magnitude of chemical shift changes for cavity protons as an evidence in support of the mode of penetration of guest into the CD cavity cannot be generalized (Table 7). In fact the mode of penetration can better be determined by the NOE experiments.

Table 7 Chemical shift changes ($\Delta\delta$) of H-3' and H-5' of β -CD protons upon complexation with selected ligands in buffered aqueous solutions at $pD = 7.0$ and $T=298.15$ K. The mode of penetration of the guest into the β -CD cavity is from wider rim side in all the cases.

Ligand	[G]/[H]	H-3'	H-5'	H-5'/H-3'
Phenethylamine	0.94	0.03	0.06	2.0
L- α -O-Benzylglycerol	0.59	0.03	0.09	3.0
1-Benzylimidazole	0.77	0.09	0.18	2.0
4-Benzylpiperidine	0.94	0.14	0.22	1.6
1-Butylimidazole	0.95	0.05	0.09	1.8
(1S, 2S)-(+)-Pseudoephedrine	0.98	0.07	0.12	1.7
(1S, 2R)-(+)-Ephedrine	1.00	0.07	0.12	1.7
(1R, 2S)-(-)-Ephedrine	0.97	0.06	0.10	1.7
Hydrocinnamate	0.92	0.04	0.10	2.5
Phenyl- β -D-glucopyranoside	0.81	0.02	0.05	2.5
3-Phenyl-1-propylamine	1.03	0.10	0.12	1.2

The formation of the CD inclusion complex also results in the concomitant chemical shift changes for the guest protons and generally all the guest protons, and not only the part included in the CD cavity, show downfield shift changes, but sometimes upfield changes are also observed.²¹⁷ These changes have mostly been found to be of qualitative significance though these may also provide important information regarding the structure of the complex.

Due to low water solubility of the guest, sometimes it is not possible to use high concentration of the guest in the NMR titrations and in such cases the chemical shift changes observed for the CD cavity protons may be very insignificant while large chemical shift changes are observed for guest protons. The chemical shift change data for

guest protons can be used for determining stoichiometry and binding constant of the complex but whether guest enters the CD cavity or not can only be said on basis of chemical shift changes in the cavity protons. ROESY experiments may be required in such cases to ascertain whether guest has actually entered the CD cavity.

Nuclear Overhauser Effect (NOE)

Beside the chemical shift changes, observed for hosts and guests, upon complexation relative to unbound state, detection of the through-space dipole-dipole interactions (NOE and/or ROESY) between the CDs and the guests is another important probe of the structure of complexes. NOEs have been used for the study of CD complexes in solution since the pioneering work of Bergeron and Rowen.²⁴⁴ Though the advent of high magnetic fields has greatly enhanced the dispersion of signals; at the same time, however, the unfavourable correlation times of complexes with molecular weights around 10^3 lead to a drop of the observed NOEs, e.g. from 34% at 90 MHz to 9% at 250 MHz. To obtain sizeable NOEs on a 400 or 500 MHz instrument, application of spin-lock techniques such as ROESY are required.²⁴⁵ In the ROESY spectra, artifacts which possess phase properties different than genuine NOE cross peaks are frequently observed between resonances within a common J-coupling network, thus being easily distinguishable. Intermolecularly, as in the case of CD complexes, these COSY-type peaks are not observed; however, false cross peaks with the same phase as genuine interactions may arise from scalar transfer from H-3' and/or H-5', the cavity protons being most prone to interact dipolarly with the guest.

The use of NOEs in the structure elucidation of CD complexes is exemplified by few representative examples. A typical NOE application is illustrated with α -CD complexes with phenol derivatives. Fig. 8 describes different inclusion modes for the cyclodextrins complexes with phenol derivatives and, obviously, all three modes should lead to quite different NOEs and should be distinguishable this way.

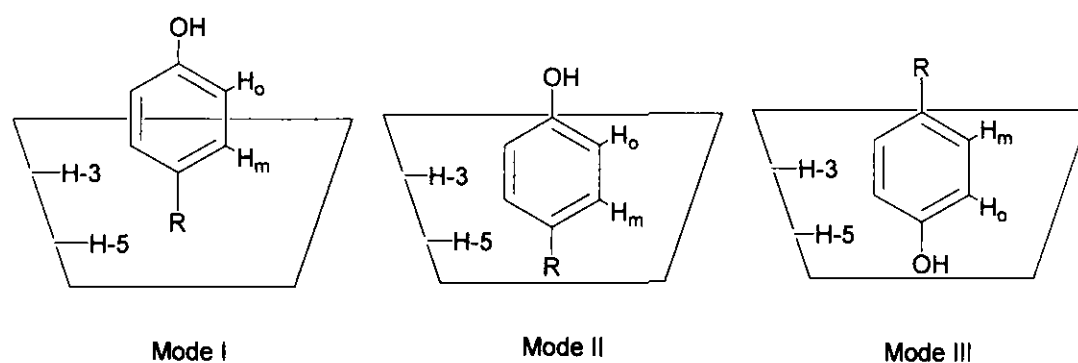


Fig. 8 Various possible modes of penetration of phenol derivatives into the CD cavity.

For Mode I with no or little immersion of the phenyl ring into the cavity there is a sizeable contact only between the guest H_m and $H-3'$ of α -CD and one can expect only small ROE at H_m upon irradiation of $H-3'$. For Mode II irradiation at $H-3'$ should lead to ROE of both H_o and H_m signals, at $H-5'$ only of the m-proton. In contrast, no effect on the H_m signal upon irradiation of $H-5'$ can be expected for Mode III. The 2D ROESY spectrum of the p-iodophenolate with α -CD (Fig 9) shows only those cross peaks discussed for Mode II.²⁴⁶

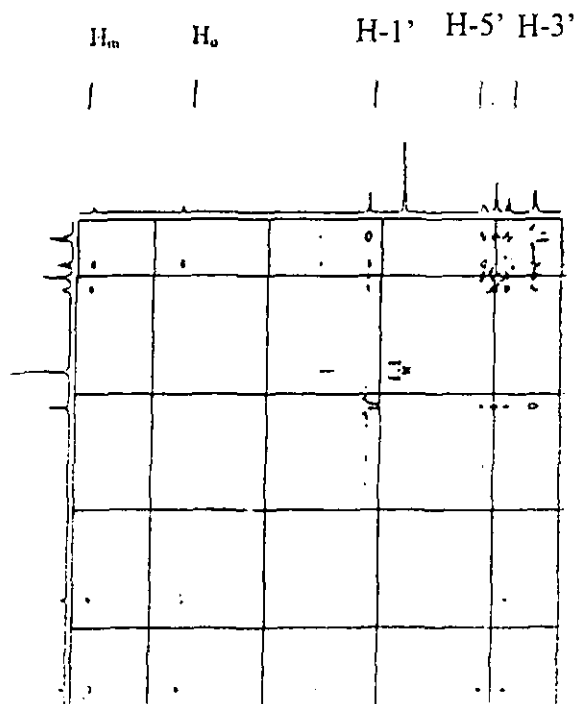


Fig. 9 Part of the ROESY spectrum showing intermolecular cross peaks between the p-iodophenolate and α -CD cavity protons supporting the inclusion Mode II.

Another interesting example is structure elucidation of a 1:1 complex between benzoic acid and β -CD.²⁴⁷ 1D ROESY experiments were performed to confirm the structure of the complex. The NOEs on the CD protons were studied on saturation of various aromatic protons. The irradiation of H_p did not show any NOE on the CD cavity protons. However, intermolecular NOEs were observed on saturation of H_o , H_m , $H-3'$ and $H-5'$. Almost equal NOE values were observed on both the cavity protons when H_o was irradiated, while the NOE value for H_m to $H-3'$ was larger than for H_m and $H-5'$. These results clearly suggest an inclusion geometry with the aromatic H_p proton located at the

CD equatorial plane, H_p on the chiral vertical axis, and H_o at similar distances from both the cavity protons. Both the geometries are compatible with this NOE data. However, NOEs on both the cavity protons upon saturation of H_m can only be explained when a fast equilibrium exists between the two inclusion complexes (Fig. 10).

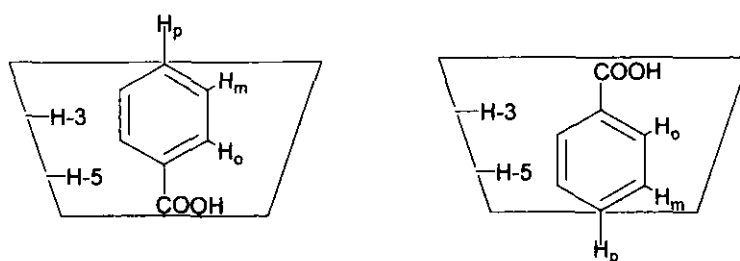


Fig. 10 Structures of two 1:1 β -CD-benzoic acid inclusion complexes.

The use of ROESY in the study of chiral recognition mechanism is exemplified by the following example. Chiral recognition of an anionic tetrahelicene (**29**) has been studied by native CDs. Fig. 11 shows the ROESY spectra of β -CD-(M)-**29** and β -CD-(P)-**29** systems in D_2O which show the different connectivities of the cavity protons with the protons of two enantiomers. It was interpreted from these results that both the CO_2^- groups of **29** are placed near the rim of the secondary OH group side of β -CD and ring A of the **29** penetrate the β -CD cavity. It was shown that there was somewhat deeper penetration of the ring A of (P)-**29** compared to that of (M)-**29** into the CD cavity.²⁴⁸

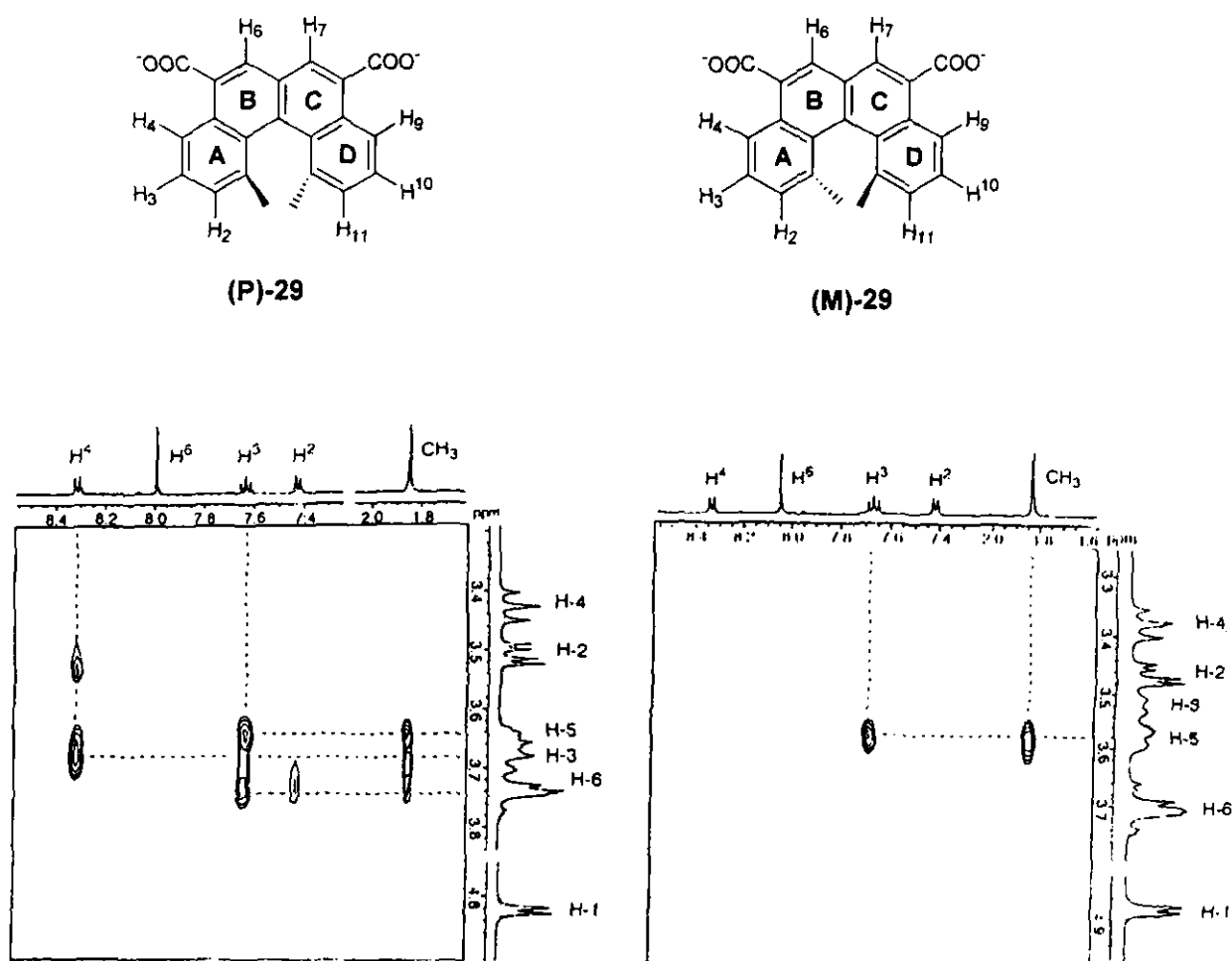


Fig. 11 ROESY spectra of (P)-29-β-CD and (M)-29-β-CD complexes.

Stoichiometry and Binding Constant of the Complex

The most commonly claimed stoichiometric ratio for CD complexes is 1:1 which is usually justified. Nevertheless, other ratios are known, most common of these probably being 2:1 (H/G) while the ratios 1:2 and 2:2 have also been reported.²³⁷

Before any structural or association constant determination is performed, it is always essential to determine the stoichiometry of the host-guest complex which is readily achieved from NMR titration data. There are several methods used to determine the stoichiometry, namely continuous variation method (Job's Plot), molar ratio method and several modified Benesi-Hildebrand methods.

Job's Plot²⁴⁹

The method of continuous variations involves preparing a series of solutions containing both the host and the guest in varying proportions so that a complete range of mole ratios is sampled ($0 < [H]/[H]+[G] < 1$) and where the total concentration $[H]+[G]$ is kept constant for each solution. The experimentally observed parameter is a host or guest chemical shift that is sensitive to complex formation. The data are plotted in the form $[H]\Delta\delta_{\text{obs}}$ versus $[H]/[H]+[G]$. The position of the maximum indicates the stoichiometry of the complex (Fig. 12).

The Mole-Ratio Method²⁵⁰

In this method a series of solutions is prepared in which the formal concentration of one of the components is held constant while that of the other is varied. A plot of the chemical shift change ($\Delta\delta$) versus mole ratio of the components is then prepared. If the formation constant is reasonably favourable, two straight lines of different slopes are obtained; the intersection occurs at a mole ratio corresponding to the combining ratio in the complex (Fig. 13).

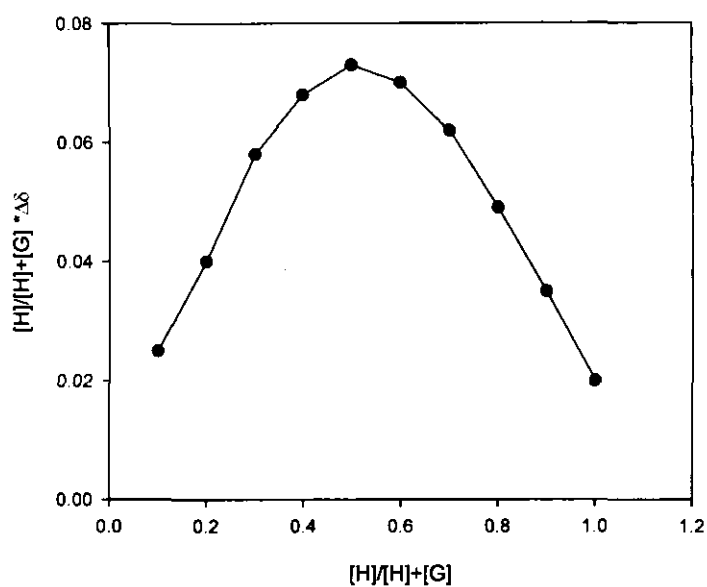


Fig. 12 A typical Job's plot for 1:1 complex.

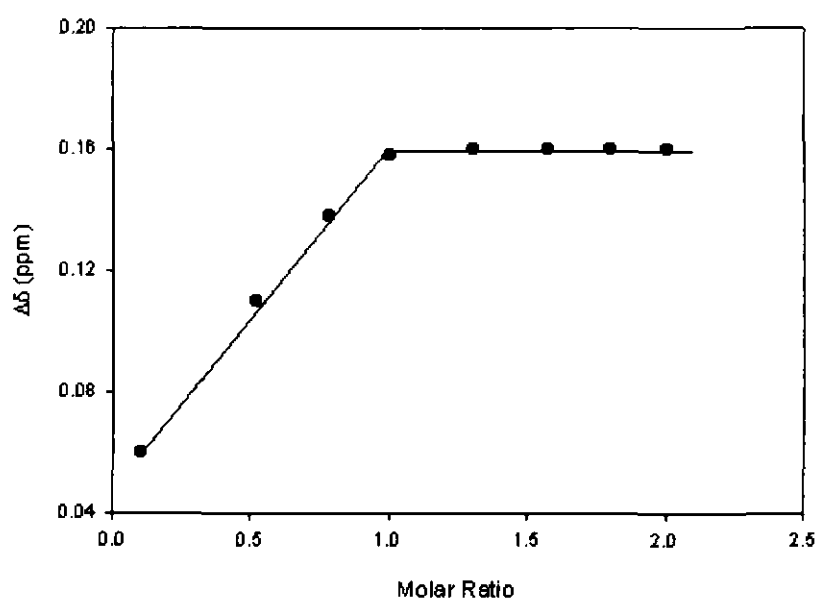


Fig. 13 A typical molar ratio plot showing 1:1 stoichiometry of the complex.

Modifications of Benesi-Hildebrand Method

The stoichiometry and stability constant of the inclusion complex can also be determined by any of the following methods. Assuming that the composition of the complex is 1:1, the following expression can be written:



The association constant of the complex (K_a) is given by

$$K_a = \frac{[\text{GH}]}{[\text{H}] [\text{G}]}$$

where [H], [G] and [GH] are equilibrium concentration of host, guest and complex, respectively. Benesi-Hildebrand studied the complexation of iodine with aromatic hydrocarbons by UV-visible spectroscopy²⁵¹ and derived the following linear equation (equation 1) for the calculation of K_a :

$$1/\epsilon_{\text{obs}} = 1/(K_a \epsilon_{\text{max}} [\text{H}]) + 1/\epsilon_{\text{max}} \quad (1)$$

where ϵ_{obs} is the extinction of a layer of solution 1 cm deep containing m moles of I_2 and ϵ_{max} is the molar extinction coefficient of the complex at the wavelength of maximum absorption. NMR version of the Benesi-Hildebrand equation was independently derived by Mathur et. al.²⁵² and Hannah and Ashbaugh²⁵³ (equation 2).

$$1/\Delta\delta_{\text{obs}} = 1/(K_a \Delta\delta_{\text{max}} [\text{H}]) + 1/\Delta\delta_{\text{max}} \quad (2)$$

where $\Delta\delta_{\text{obs}} = (\delta_{\text{G}} - \delta_{\text{obs}})$ and $\Delta\delta_{\text{max}} = (\delta_{\text{G}} - \delta_{\text{GH}})$.

A plot of $1/\Delta\delta_{\text{obs}}$ against $1/[\text{H}]$ (often referred to as a double reciprocal plot) should be linear for a 1:1 complex, with a slope $1/K_{\text{a}}\Delta\delta_{\text{max}}$ and intercept $1/\Delta\delta_{\text{max}}$ allowing the determination of stability constant (K_{a}). This expression is valid when observing one species in presence of a large excess of the other species.

An alternative solution of Benesi-Hildebrand equation has been proposed by Foster and Fyfe (equation 3).²⁵⁴

$$\Delta\delta_{\text{obs}}/[\text{H}] = -K_{\text{a}} \Delta\delta_{\text{obs}} + K_{\text{a}} \Delta\delta_{\text{max}} \quad (3)$$

This is a special form of the more general Scatchard plot.²⁵⁵ In the Foster-Fyfe procedure, a plot of $\Delta\delta_{\text{obs}}/[\text{H}]$ against $\Delta\delta_{\text{obs}}$ (referred as an x-reciprocal plot) should be linear for a 1:1 complex. The gradient is equal to $-K_{\text{a}}$ and the intercept gives $\Delta\delta_{\text{max}}$. This modification requires an extrapolation to infinitely dilute solution and the K_{a} is not dependent on the extrapolation.

Another modification of Benesi-Hildebrand equation is Scott equation (equation 4).²⁵⁶

$$[\text{H}]/\Delta\delta_{\text{obs}} = [\text{H}]/\Delta\delta_{\text{max}} + \Delta\delta_{\text{max}}/K_{\text{a}} \quad (4)$$

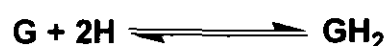
In the Scott procedure, a plot of $[\text{H}]/\Delta\delta_{\text{obs}}$ is plotted against $[\text{H}]$ (referred to as a y-reciprocal plot) which should be linear for a 1:1 complex with a slope $1/\Delta\delta_{\text{max}}$ and intercept $\Delta\delta_{\text{max}}/K_{\text{a}}$ allowing the estimation of stability constant (K_{a}).

The stoichiometry and stability constant of the 1:1 tetramethrin and β -CD inclusion complex were determined by spectrofluorometry²⁵⁷ through double reciprocal plot (equation 5).²⁵⁸

$$1/\Delta F_{\text{obs}} = 1/(K_a \Delta F_{\text{max}} [H]) + 1/\Delta F_{\text{max}} \quad (5)$$

where ΔF_{obs} denotes the change in fluorescence intensity of tetramethrin in the presence of β -CD compared to pure tetramethrin and ΔF_{max} change in fluorescence intensity of tetramethrin, when all of its molecules are complexed with β -CD, compared to pure tetramethrin while other symbols have their usual meaning. The equation is identical to the Hannah and Ashbaugh modification (equation 2) except that a different property of the complex is studied.

Now, assuming the complex to be 1:2, the following expression can be written:



$$K_a = \frac{[GH_2]}{[H]^2 [G]}$$

if $[H] \gg [GH_2] \gg [GH]$ then the following linear expression (equation 6) is obtained for calculating stability constant by fluorospectrometry.

$$1/\Delta F_{\text{obs}} = 1/(K_a \Delta F_{\text{max}} [H]^2) + 1/\Delta F_{\text{max}} \quad (6)$$

where symbols have their usual meaning. A plot of $1/\Delta F_{\text{obs}}$ against $1/[H]^2$ gives a straight line for a 1:2 complex. Thus, the NMR version of this equation can be written as:

$$1/\Delta\delta_{\text{obs}} = 1/(K_a\Delta\delta_{\text{max}}[\text{H}]^2) + 1/\Delta\delta_{\text{max}} \quad (7)$$

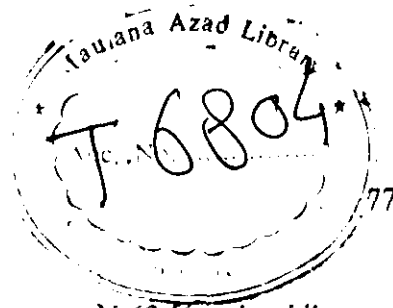
A plot of $1/\Delta\delta_{\text{obs}}$ verses $1/[\text{H}]^2$ should be a straight line for 1:2 complex with a slope $1/K_a\Delta\delta_{\text{max}}$ and intercept $1/\Delta\delta_{\text{max}}$ allowing the determination of stability constant.

These are conditional values in which activity coefficients are not considered.

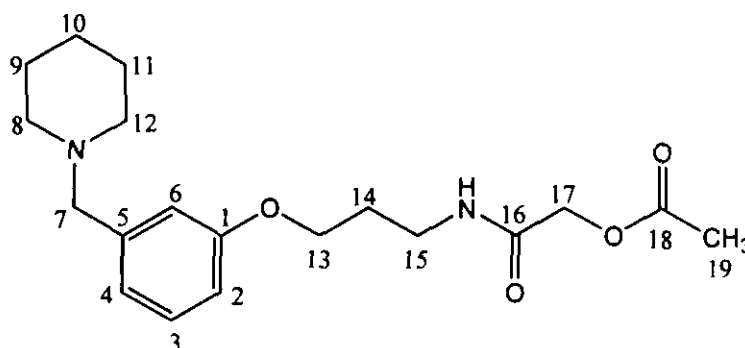
Throughout the above discussion it is assumed that the guest molecule is the observed species. It does not matter which species is observed and the most readily observed and responsive molecule would normally be chosen. The data treatment for observed host is identical, with host and guest symbols switched.

Throughout discussion the CD protons are numbered as H-1' to H-6'.

*¹H NMR Spectroscopic Study of
Complexation of Roxatidine Acetate
Hydrochloride with β -Cyclodextrin*



Roxatidine acetate hydrochloride (RAH), chemically known as N-{3-[(α -piperidino-m-tolyl)oxy]propyl} glycolamide acetate mono hydrochloride, is a histamine H₂ receptor antagonist.²⁵⁹ The drug is recommended for the management of benign and post operative ulcer, as it does not appear to affect cytochrome P450 and is therefore considered to have little effect on metabolism of other drugs.²⁶⁰



Roxatidine acetate

Experimental

^1H NMR spectra of pure RAH (Fig. 14) and five mixtures of RAH and β -CD (Figs. 15-19) were recorded in D_2O at room temperature and the chemical shift values were recorded in δ (ppm). ^1H NMR spectra for pure RAH and two mixtures (A, B) were recorded on a 200 MHz instrument while those for three mixtures (C-E) were recorded on a 300 MHz instrument. The chemical shift change data for β -CD protons was obtained, relative to reported data,²³⁷ by keeping the concentration of β -CD constant while varying the concentration of RAH. The molar ratios ($[\text{RAH}]/[\beta\text{-CD}]$) were calculated by direct integration of appropriate signals. All the spectra consisted of one set of concentration dependent resonances for each proton or group of equivalent protons indicating a fast reversible exchange between free and complexed RAH on the NMR time scale.

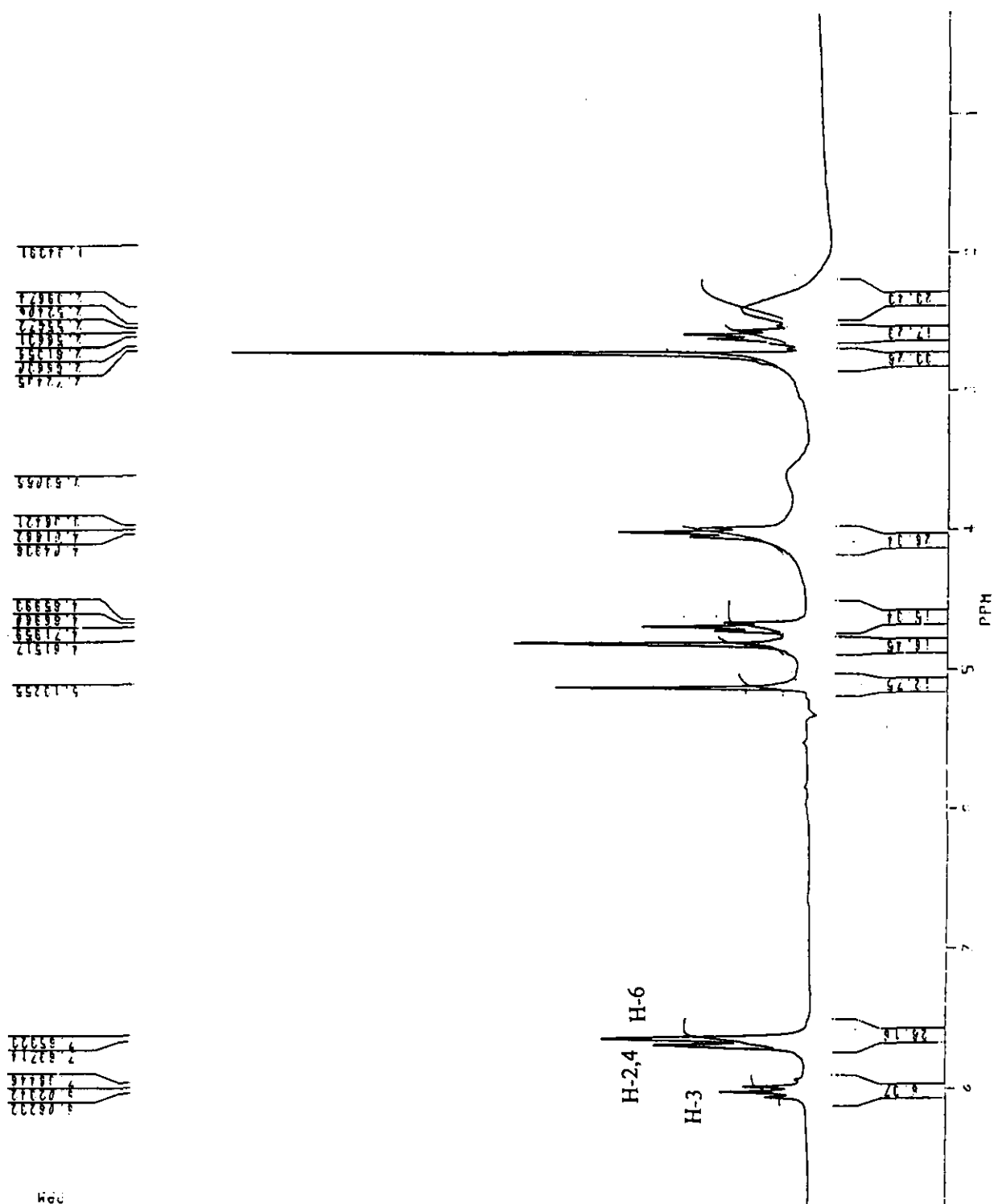


Fig. 14 ¹H NMR spectrum (200 MHz) of pure roxatidine acetate hydrochloride.

Fig. 15 ^1H NMR spectrum (200 MHz) of a mixture of roxatidine acetate hydrochloride and β -CD having a molar ratio ($[\text{RAH}]/[\beta\text{-CD}]$) equal to 0.77.

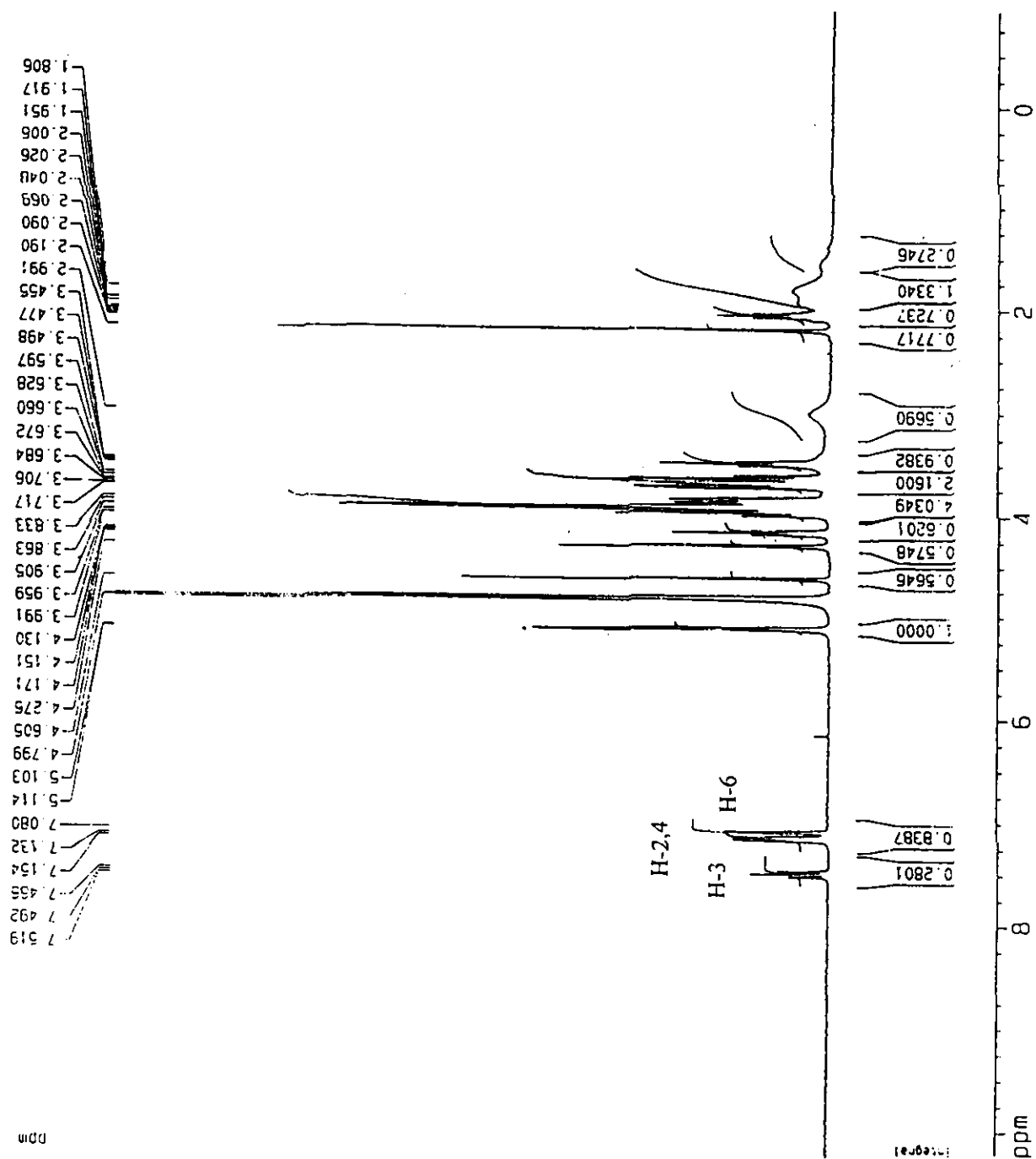


Fig. 17 ^1H NMR spectrum (200 MHz) of a mixture of roxatidine acetate hydrochloride and β -CD having a molar ratio ($[\text{RAH}]/[\beta\text{-CD}]$) equal to 1.96.

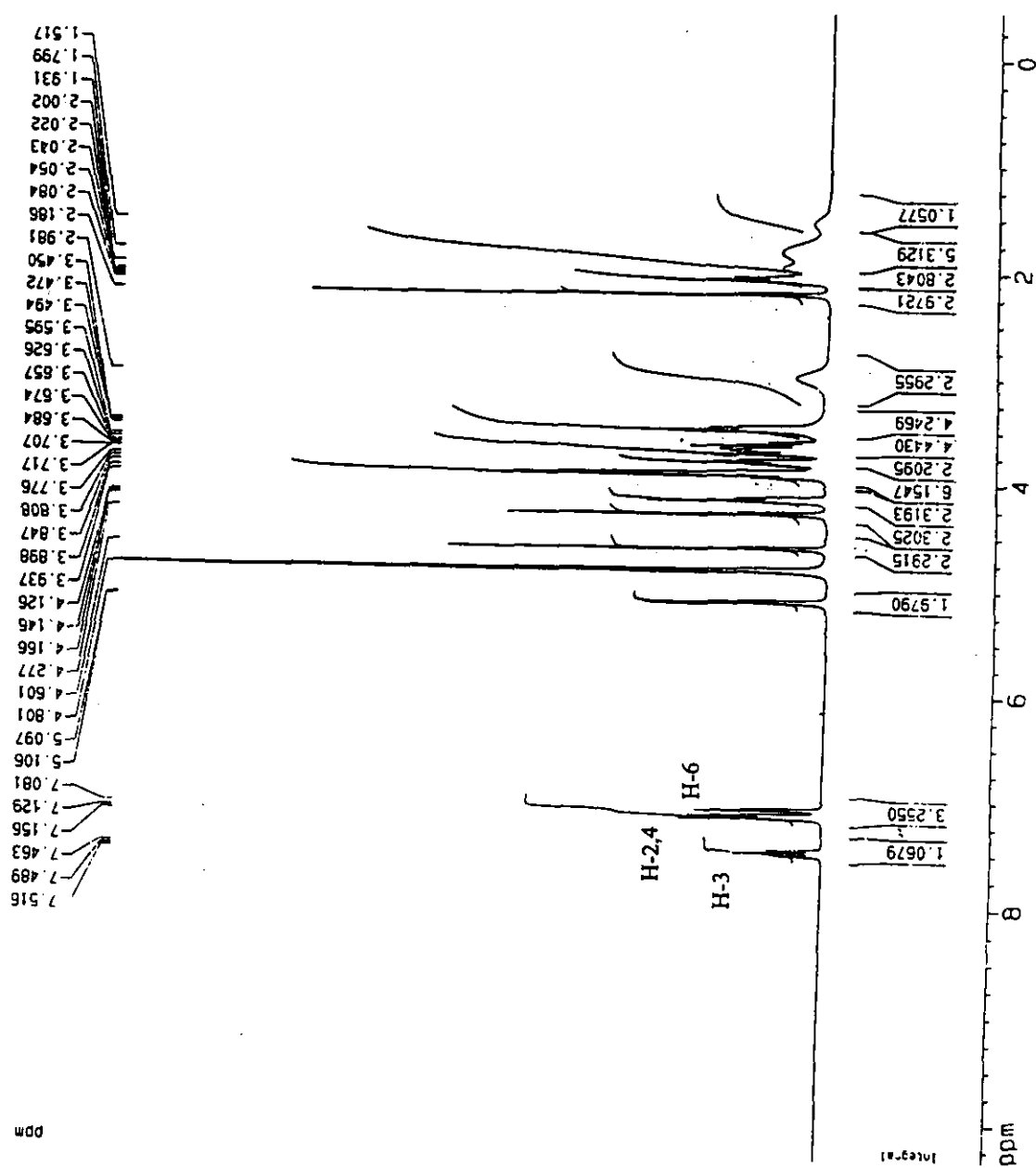


Fig. 18 ^1H NMR spectrum (200 MHz) of a mixture of roxatidine acetate hydrochloride and β -CD having a molar ratio ($[\text{RAH}]/[\beta\text{-CD}]$) equal to 3.50.

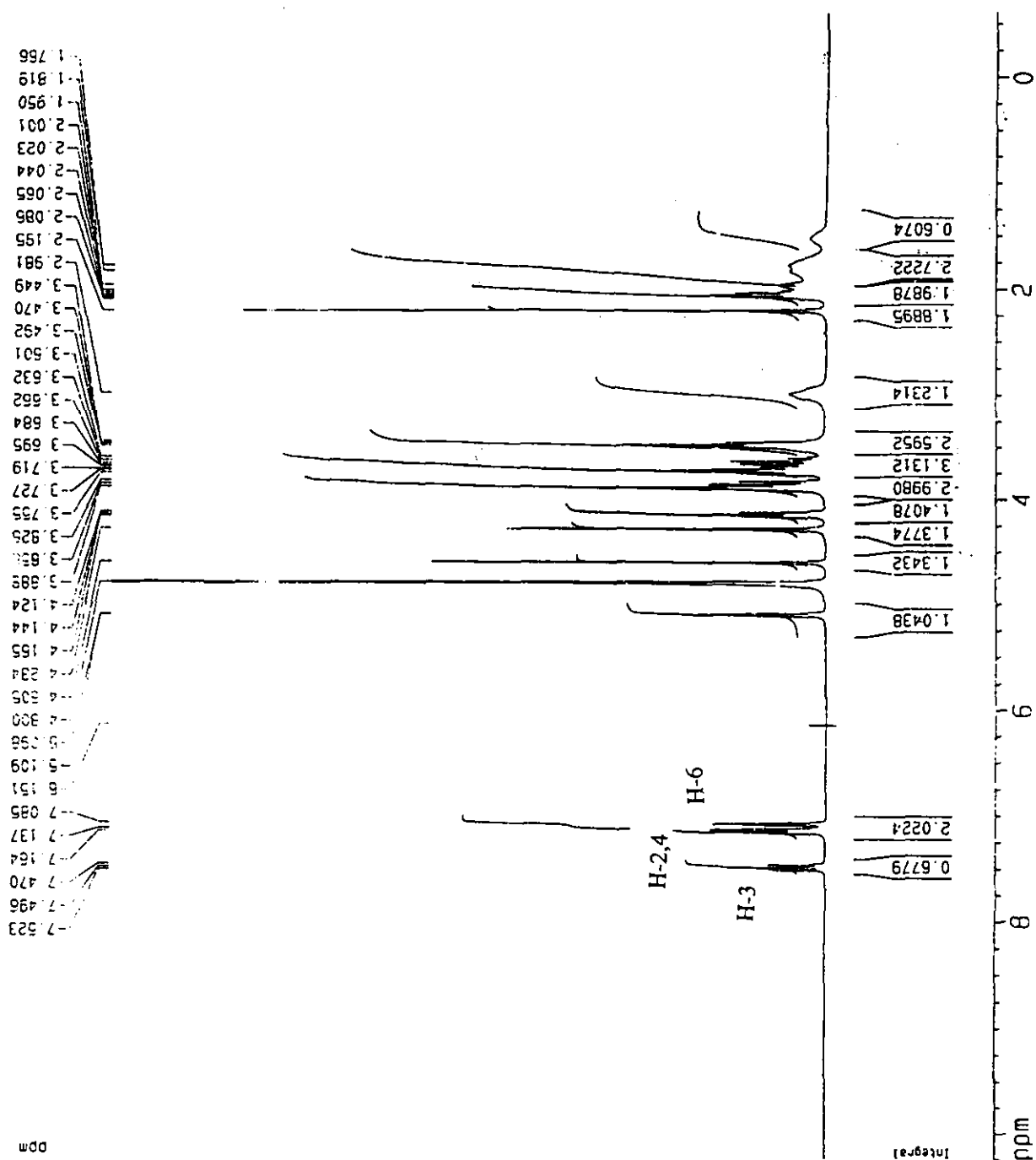


Fig. 19 ^1H NMR spectrum (200 MHz) of a mixture of roxatidine acetate hydrochloride and β -CD having a molar ratio ($[\text{RAH}]/[\beta\text{-CD}]$) equal to 4.55.

Result and Discussion

^1H NMR Chemical Shift Change Data of β -CD

The protons situated in the β -CD cavity, namely H-3' and H-5', exhibited significant highfield shift changes in the presence of RAH which increased with the increasing concentration of RAH while other β -CD protons did not show any significant chemical shift changes. Fig. 20 shows changes in the chemical shifts of β -CD protons in the presence of RAH compared to pure β -CD while the ^1H NMR chemical shift change data for all the β -CD protons is given in Table 8. The highfield shift changes in the β -CD cavity protons confirmed the inclusion of the RAH into the β -CD cavity resulting in the formation of RAH- β -CD complex, as reported earlier.^{233, 237, 261, 262}

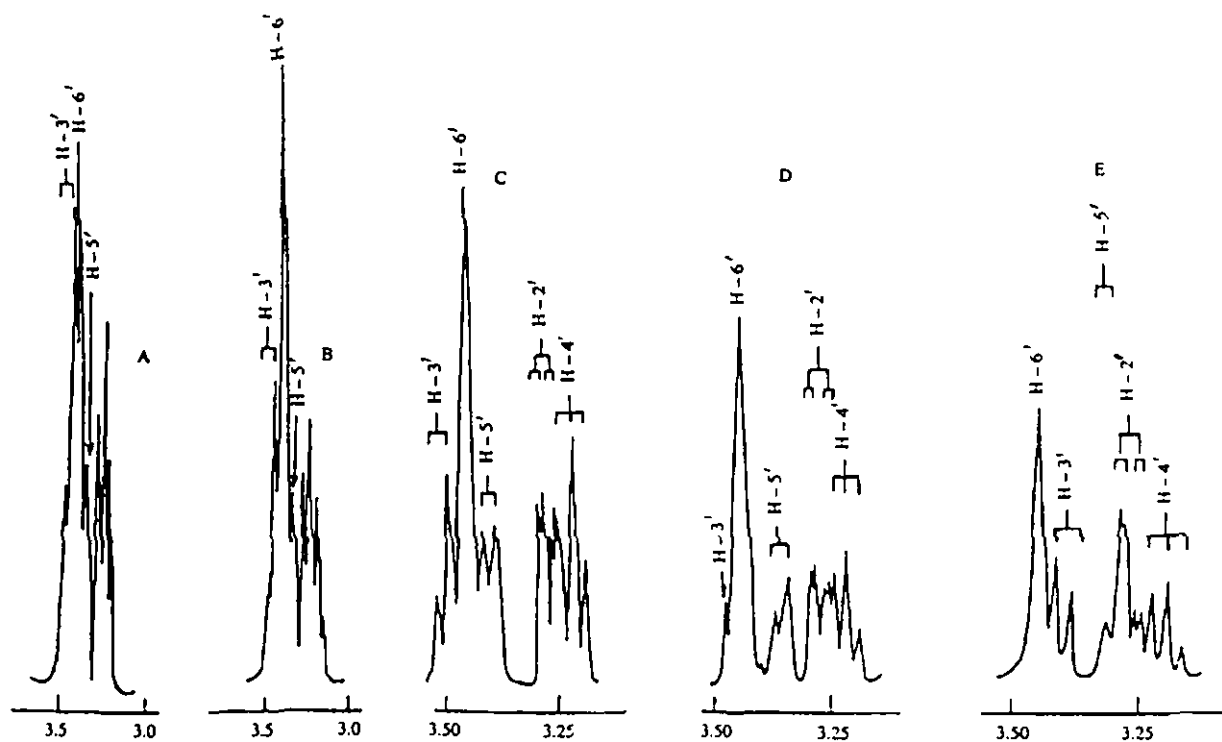


Fig. 20 Part of the ^1H NMR spectra of mixtures of RAH and β -CD showing β -CD protons. (Temperature = 25 $^{\circ}\text{C}$, Solvent = D_2O)

Table 8 ^1H NMR chemical shift change data ($\Delta\delta$) of β -CD protons, in the presence of RAH.
(Temperature = 25 °C, Solvent = D_2O)

Sample	[RAH]/ [β -CD]	H-2'	H-3'	H-4'	H-5'	H-6'	$\Delta\delta_{\text{H-5'}}$ / $\Delta\delta_{\text{H-3'}}$
A	0.77	0.0141	-0.0176	0.0093	-0.0182	-0.0075	1.03
B	1.19	0.0107	-0.0276	0.0079	-0.0281	-0.0095	1.01
C	1.96	0.0060	-0.0495	0.0095	-0.0505	-0.0135	1.02
D	3.50	0.0140	-0.0985	0.0145	-0.0995	-0.0135	1.01
E	4.55	0.0170	-0.1455	0.0185	-0.1535	-0.0255	1.05

Negative values indicate highfield shift

Stoichiometry of the RAH- β -CD Complex

The stoichiometry of the RAH- β -CD complex was determined by the Scott modification²⁵⁶ of Benesi-Hildebrand method.²⁵¹ The ^1H NMR shift change data for β -CD protons was obtained, in the presence of RAH, by keeping the concentration of β -CD constant while varying the concentration of RAH. A plot of the chemical shift change data for H-3' of β -CD against [RAH] in the form of $([\text{RAH}]/[\beta\text{-CD}])/\Delta\delta$ verses $([\text{RAH}]/[\beta\text{-CD}])$ gave excellent linear fit (Fig. 21) supporting the 1:1 stoichiometry of the RAH- β -CD complex.

^1H NMR Assignment of Roxatidine Acetate Hydrochloride

The signals of RAH and β -CD did not interfere with each other in any of the spectrum. The signals for aromatic protons appeared as a singlet (H-6), a doublet ($J = 8.1$ Hz, H-2, 4) and a triplet ($J = 8.0$ Hz, H-3) in all the cases except in the spectrum of pure drug in which the doublet for H-2, 4 and singlet for H-6 were found partly overlapping. The

assignment of the non-aromatic protons could not be done due to poor resolution of the signals and these were therefore not studied.

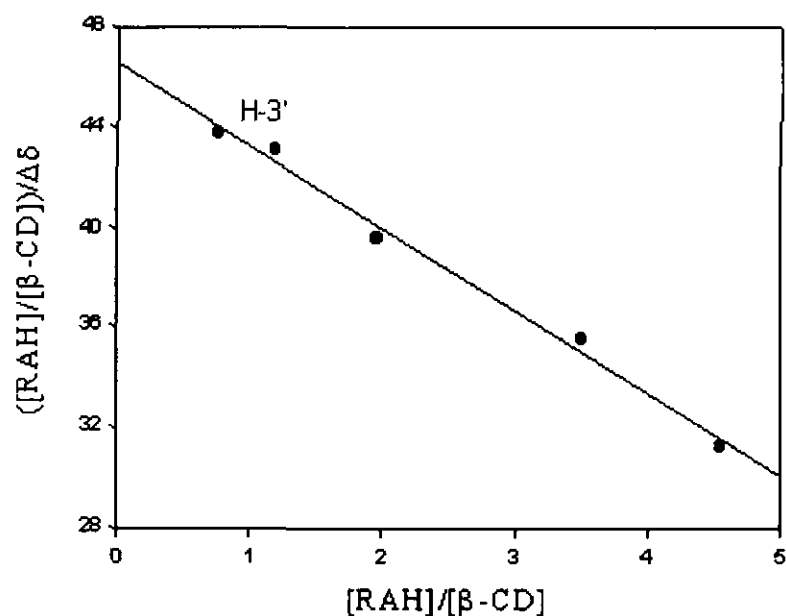


Fig. 21 Scott plot showing 1:1 stoichiometry for RAH-β-CD inclusion complex.

¹H NMR Chemical Shift Change Data of Roxatidine Acetate Hydrochloride

As expected, changes in the shapes as well as chemical shifts of RAH protons were observed in the presence of β-CD. All the protons, except the aromatic proton H-6, exhibited downfield shift changes. There were no significant changes observed in the chemical shift values for RAH in the spectra of samples C, D and E. The chemical shift change (Δδ) data for all the aromatic protons of RAH is given in Table 9.

Table 9 ^1H NMR chemical shift change ($\Delta\delta$) data for RAH aromatic protons in the presence of β -CD in D_2O . (Temperature = 25°C)

Sample	[RAH]/[β -CD]	H-2,4	H-3	H-6
A	0.77	0.0214	0.0302	-0.0388
B	1.19	0.0155	0.0220	-0.0322
C	1.96	0.0100	0.0120	-0.0130
D	3.50	0.0070	0.0082	-0.0087
E	4.55	0.0050	0.0041	-0.0037

Negative values indicate highfield shift

It can be concluded on the basis of chemical shift changes in the proton resonances of β -CD and RAH compared to those of pure samples, in solution, in conjunction with the 1:1 stoichiometry of the complex that RAH forms a 1:1 inclusion complex with β -CD by the shallow penetration of probably aromatic ring, due to the presence of two bulky groups, into the β -CD cavity. It can not be said with certainty, however, which part of the guest is entering the β -CD cavity and also whether the penetration of the guest is from wider or narrower rim side of the cavity. The structure for the RAH- β -CD complex could not, therefore, be confirmed.

We studied the complexation of RAH with β -CD again because the resolution in the 200 MHz spectra was not good and there was some element of doubt in the calculation of chemical shift changes.

Experimental

All the NMR spectra were recorded on a 500 MHz instrument. ^1H NMR spectra of RAH, in the absence (Fig. 22) as well as in the presence of β -CD (Figs. 23-28), were recorded in D_2O at room temperature. The chemical shift values are reported in δ (ppm) with reference to HDO peak. To determine the stoichiometry and binding constant of the complex the chemical shift change data for β -CD protons was obtained by keeping the concentration of β -CD constant at 10 mM while varying the concentration of RAH from 2.11 to 36.7 mM. COSY spectrum was recorded for pure RAH (Fig 29) while ROESY and NOESY spectra were obtained for a mixture of RAH and β -CD. All the spectra consisted of one set of concentration dependent resonances for each proton or group of equivalent protons indicating a fast reversible exchange between free and complexed RAH on the NMR time scale.

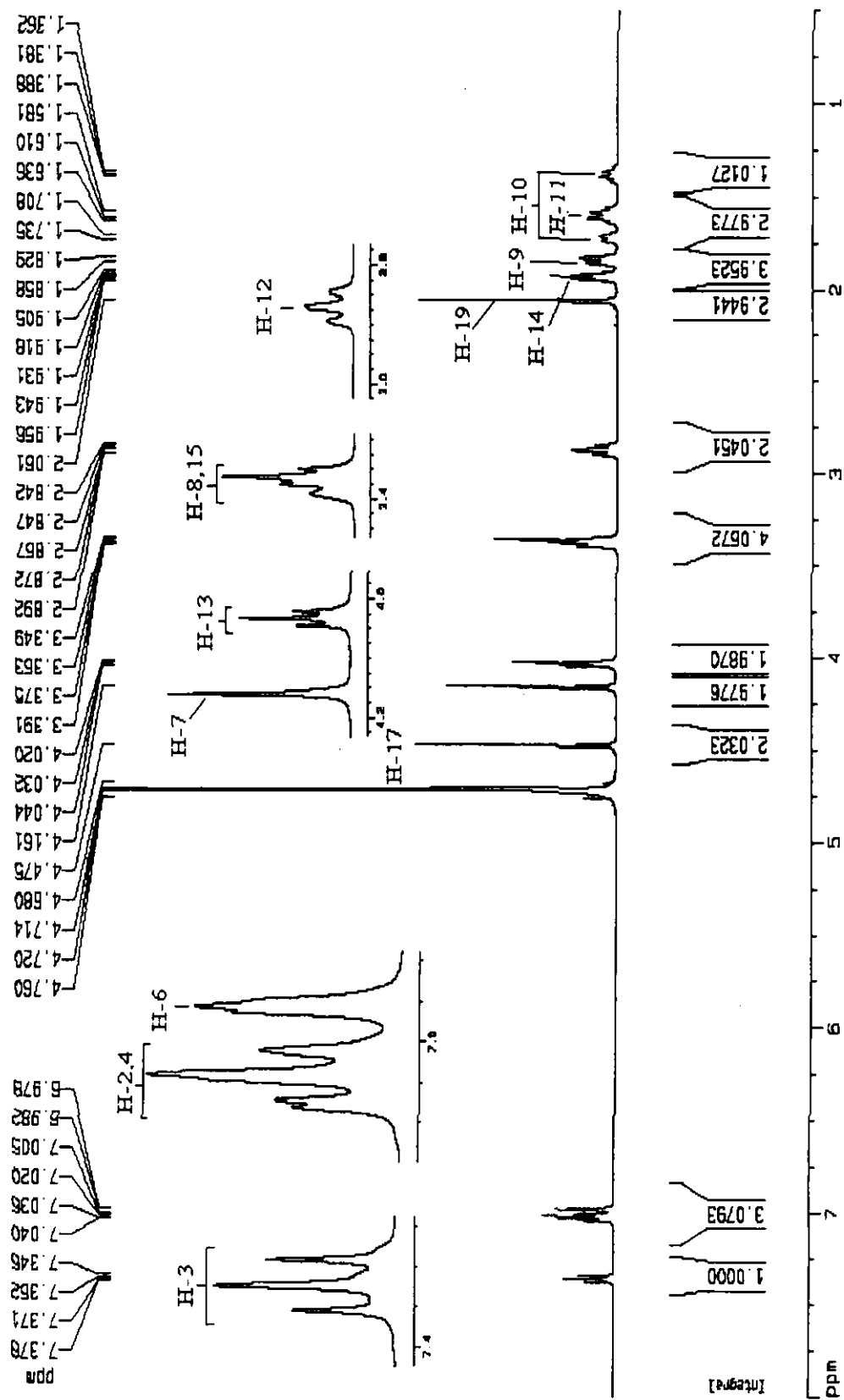


Fig. 22 ^1H NMR spectrum of pure roxatidine acetate hydrochloride.

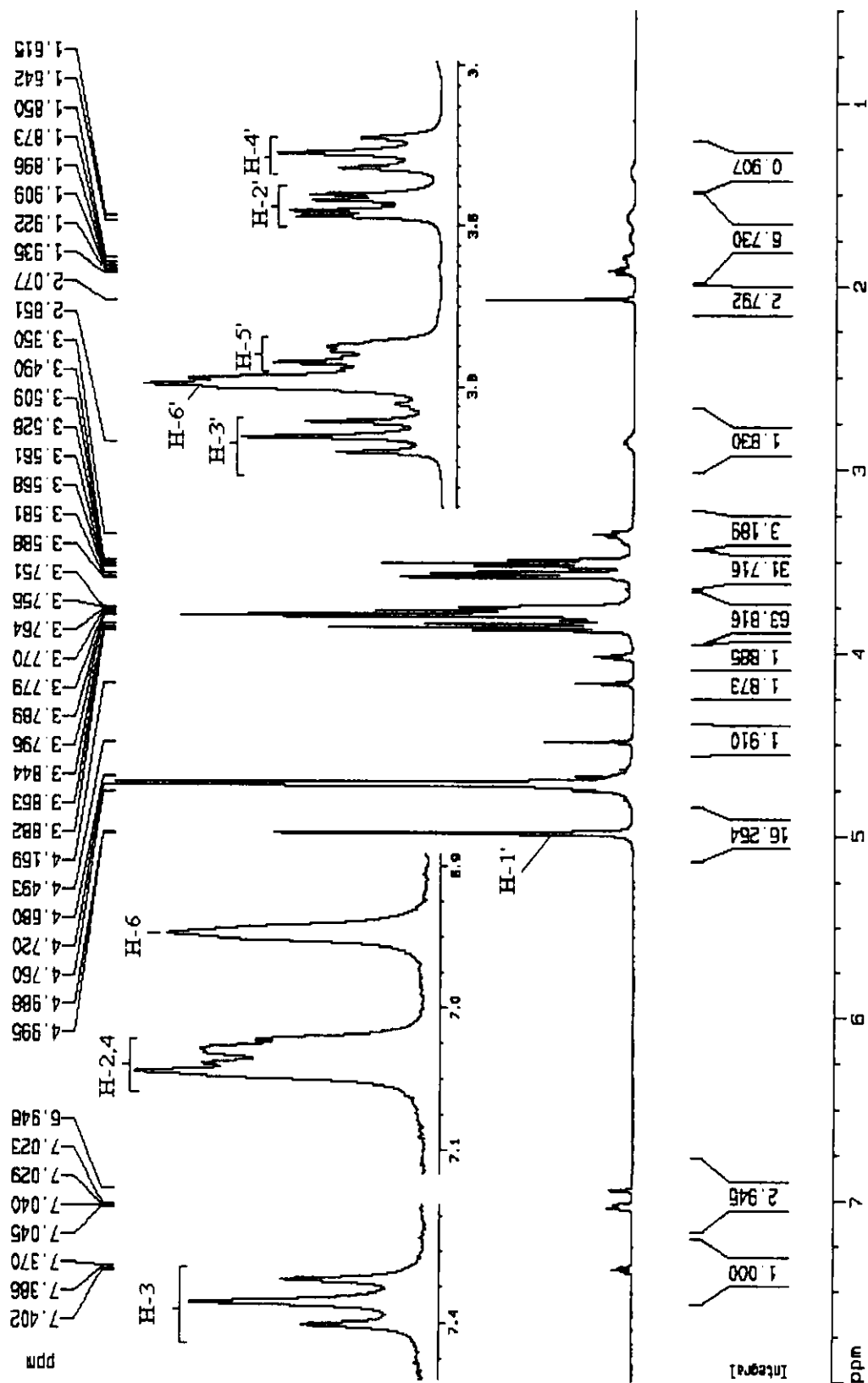


Fig. 23 ¹H NMR spectrum of a mixture of roxatidine acetate hydrochloride and β-CD having a molar ratio ([β-CD]/[RAH]) equal to 2.367.

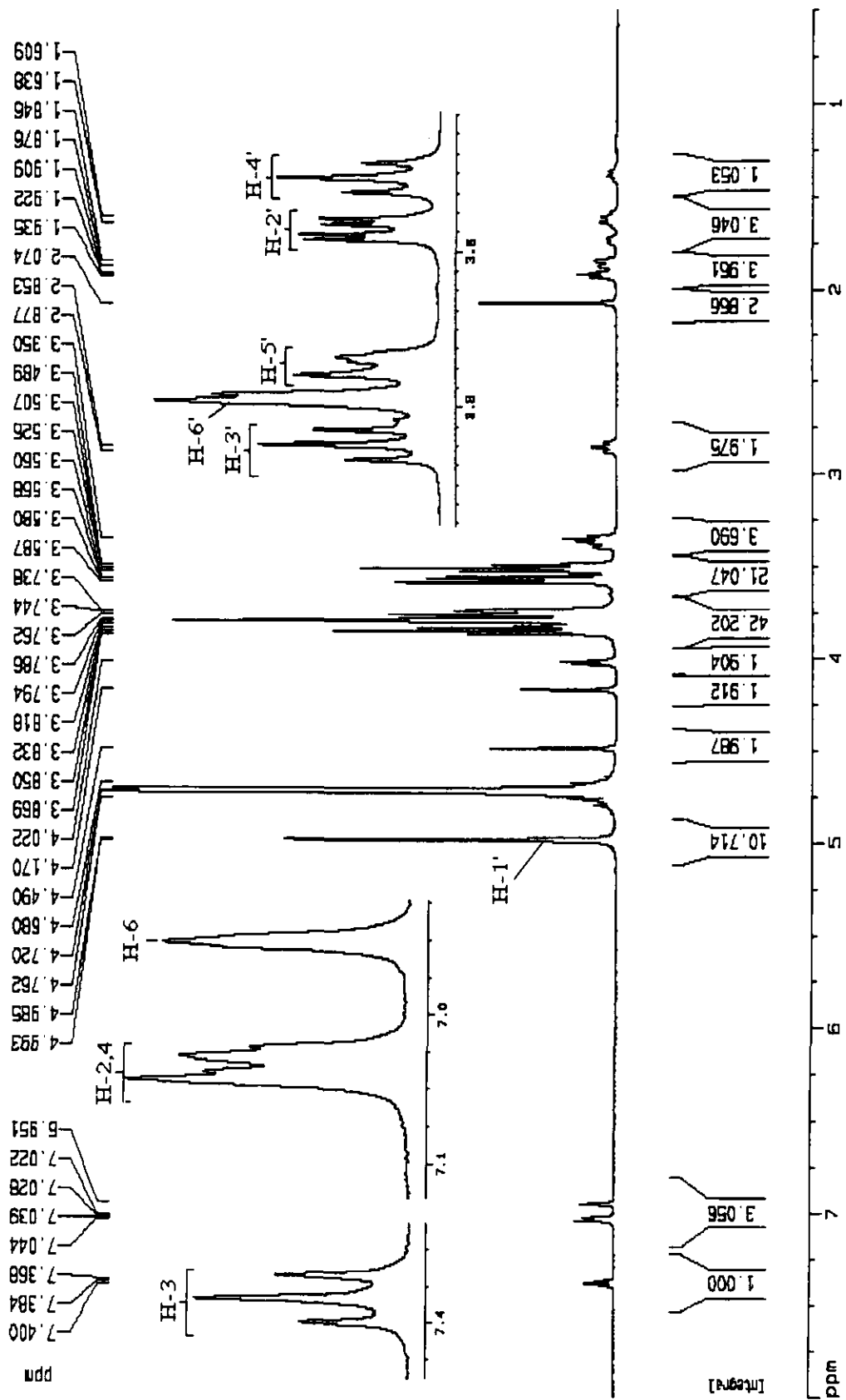


Fig. 24 ^1H NMR spectrum of a mixture of roxatidine acetate hydrochloride and β -CD having a molar ratio ($[\beta\text{-CD}]/[\text{RAH}]$) equal to 1.503.

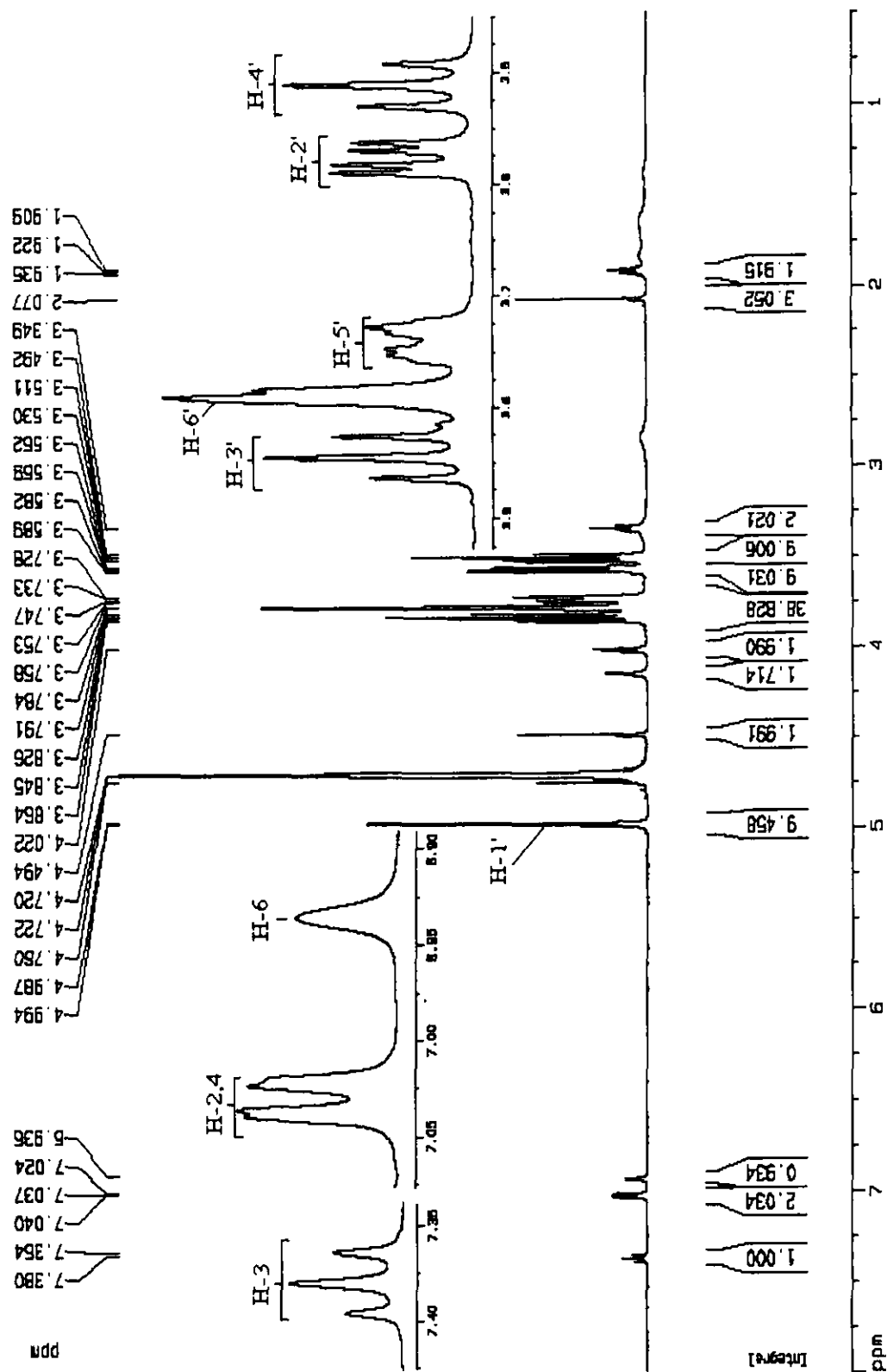


Fig. 25 ^1H NMR spectrum of a mixture of roxatidine acetate hydrochloride and β -CD having a molar ratio ($[\beta\text{-CD}]/[\text{RAH}]$) equal to 1.366.

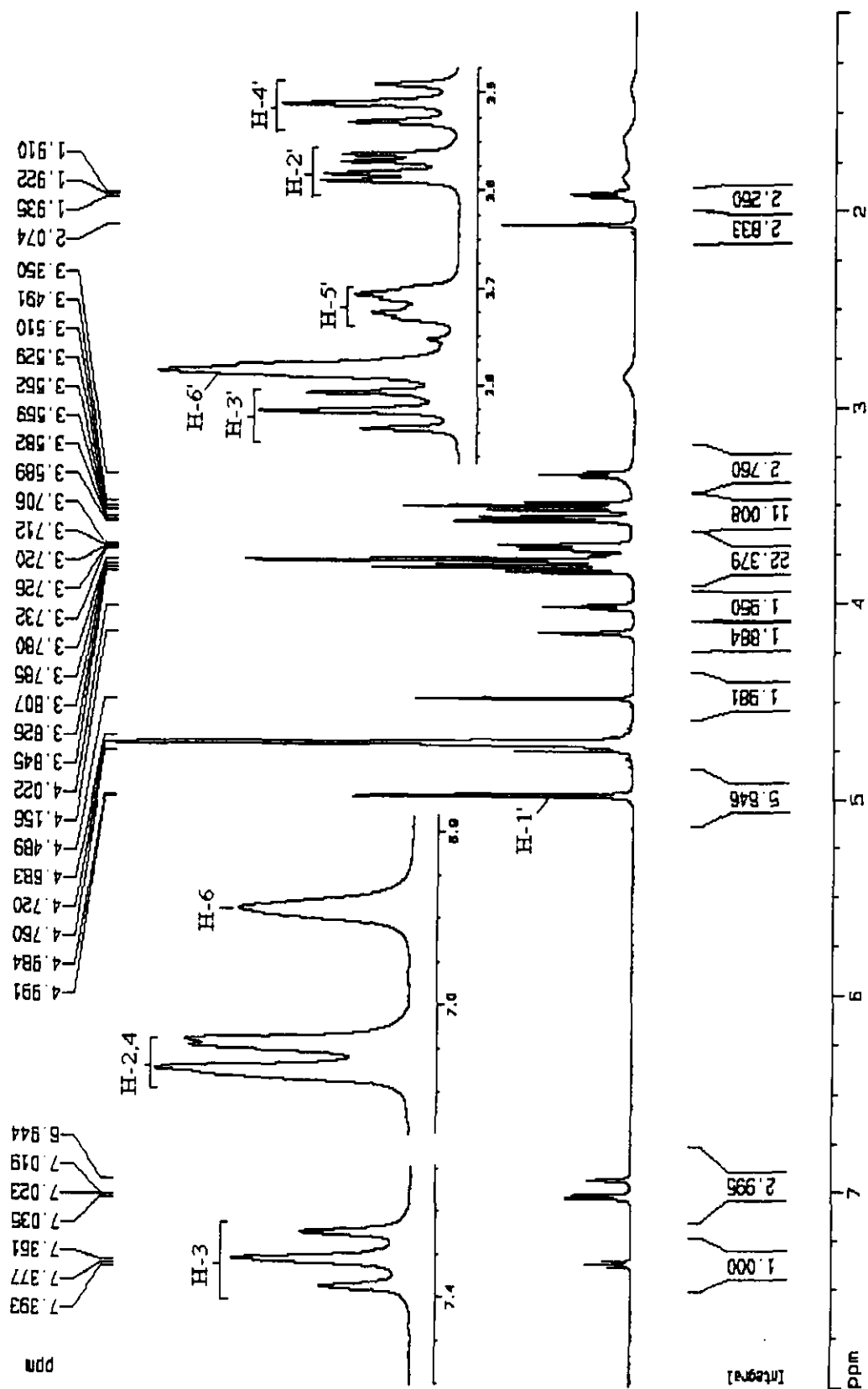


Fig. 26 ^1H NMR spectrum of a mixture of roxatidine acetate hydrochloride and β -CD having a molar ratio $[\beta\text{-CD}]/[\text{RAH}]$ equal to 0.808.

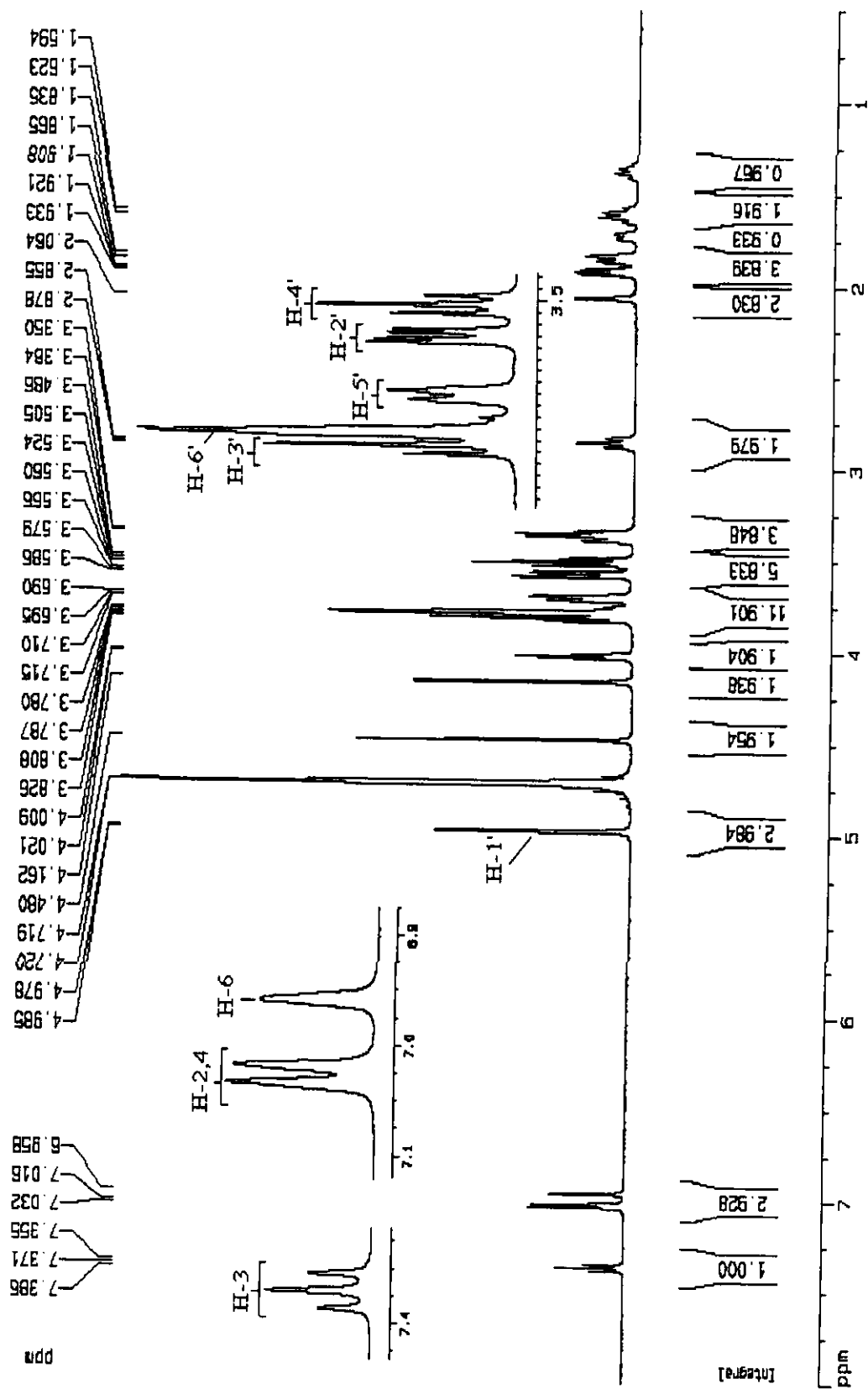


Fig. 27 ^1H NMR spectrum of a mixture of roxatidine acetate hydrochloride and β -CD having a molar ratio $([\beta\text{-CD}]/[\text{RAH}])$ equal to 0.436.

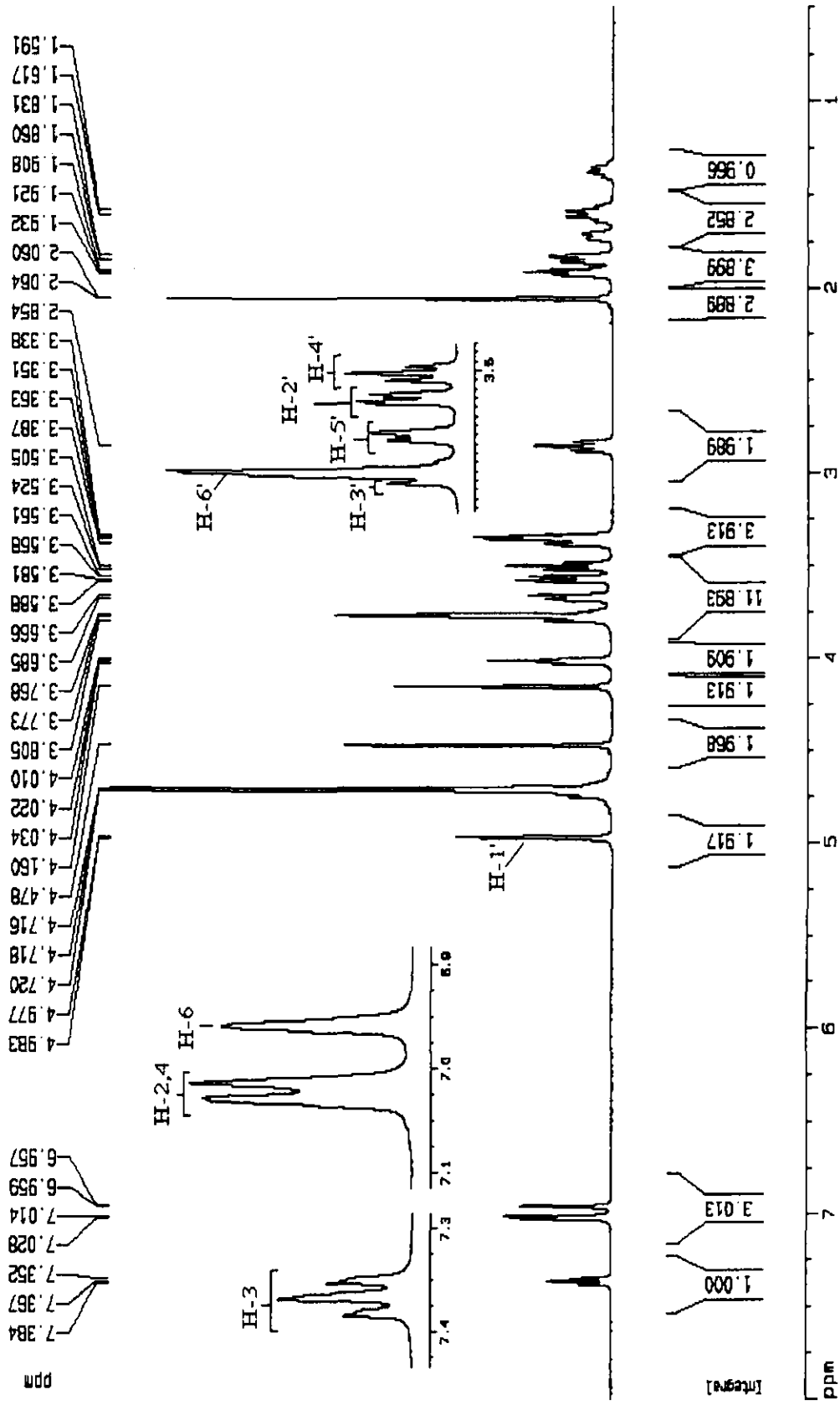


Fig. 28 ^1H NMR spectrum of a mixture of roxatidine acetate hydrochloride and β -CD having a molar ratio ($[\beta\text{-CD}]/[\text{RAH}]$) equal to 0.272.

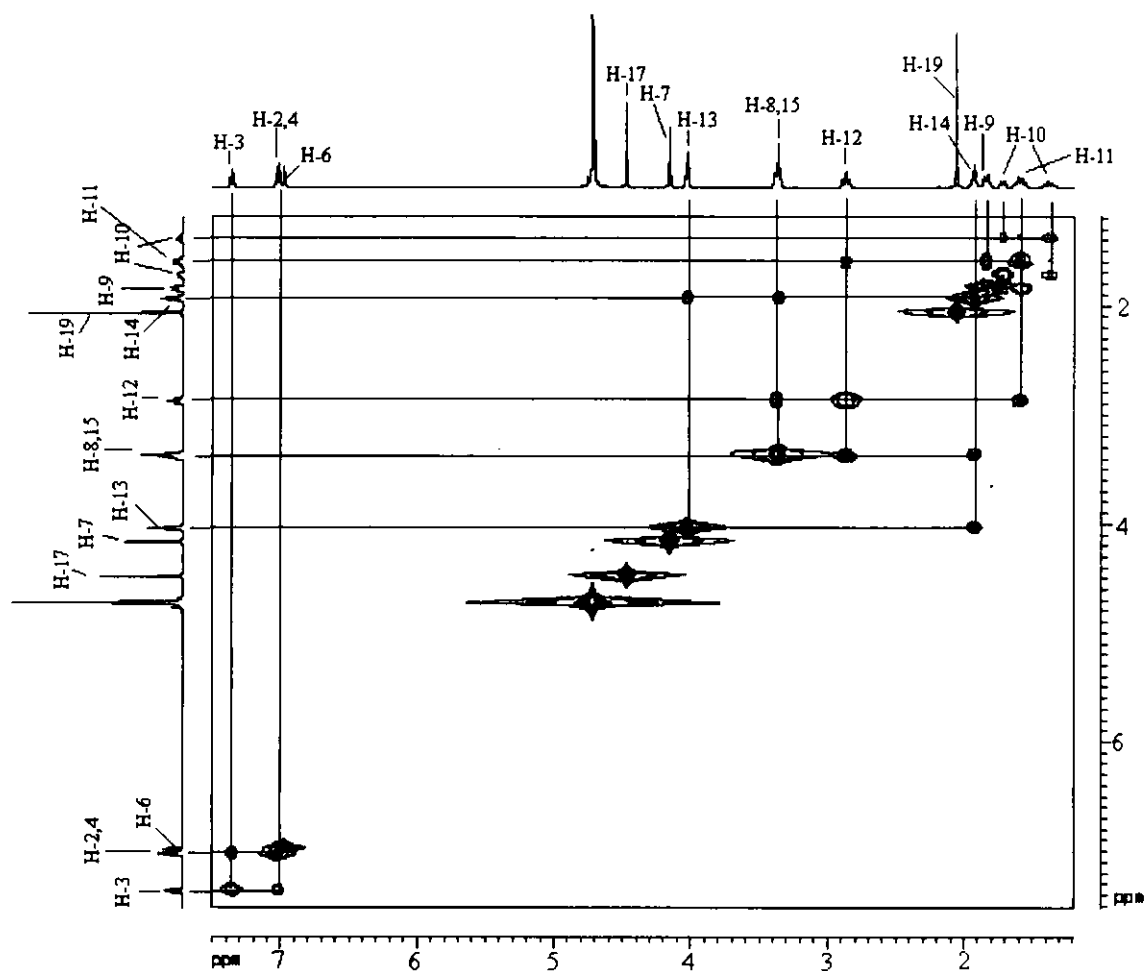


Fig. 29 2D COSY spectrum of pure RAH.

Result and Discussion

¹H NMR Chemical Shift Change Data of β -CD

All the signals for β -CD protons were easily identified by their characteristic shapes and chemical shifts values. In the presence of RAH, β -CD cavity protons positioned inside the β -CD cavity, namely H-3' and H-5', exhibited highfield shift changes which increased with the increase in the concentration of RAH while other protons remained more or less unaffected. The chemical shift change data for all the β -CD protons is given in Table 10 and the gradual highfield shifts of the β -CD cavity protons with the increase in the concentration of RAH are shown in Fig. 30. These shift changes in the H-3' and H-5' protons of β -CD can only be explained in terms of ring current effect of aromatic ring included in the cavity, thus confirming the formation of inclusion complex between RAH and β -CD as reported earlier.

Table 10 ¹H NMR chemical shift change data ($\Delta\delta$) for β -CD protons in the presence of RAH. (Temperature = 25 °C, Solvent = D₂O)

[β-CD]/[RAH]	H-1'	H-2'	H-3'	H-4'	H-5'	H-6'
2.367	0.008	0.121	-0.014	0.009	-0.004	0.002
1.503	0.006	0.011	-0.027	0.008	-0.015	-0.001
1.366	0.007	0.013	-0.032	0.012	-0.018	-0.003
0.808	0.004	0.013	-0.051	0.010	-0.046	-0.008
0.436	-0.001	0.011	-0.069	0.006	-0.062	-0.011
0.272	-0.003	0.012	-0.089	0.006	-0.089	-0.023

Negative values indicate highfield shift.

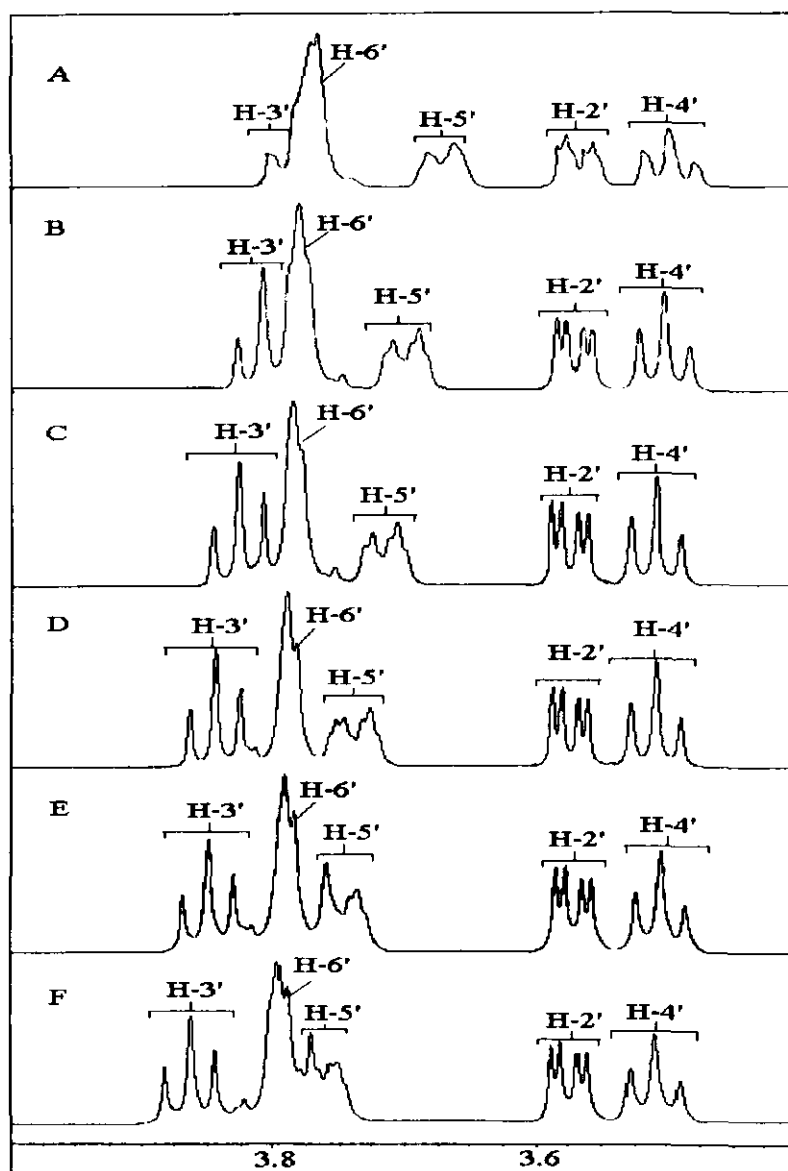


Fig. 30 Part of ^1H NMR spectra (500 MHz) showing β -CD proton signals of mixtures of β -CD and RAH having molar ratios ($[\beta\text{-CD}]/[\text{RAH}]$) (A) 2.367 (B) 1.503 (C) 1.366 (D) 0.808 (E) 0.436 (F) 0.272. (Temperature = 25 $^\circ\text{C}$, Solvent = D_2O)

Stoichiometry of the RAH- β -CD Complex

The stoichiometry of the RAH- β -CD complex was determined by the Scott equation for 1:1 complex.²⁵⁶

$$[G]/\Delta\delta_{\text{obs}} = [G]/\Delta\delta_{\text{max}} + \Delta\delta_{\text{max}}/K_a$$

The ^1H NMR chemical shift change ($\Delta\delta$) data for β -CD protons was obtained, in the presence of RAH, by keeping the concentration of β -CD constant while varying the concentration of RAH. The plot of $\Delta\delta_{\text{H-5'}}$ against $[\text{RAH}]$ in the form of $[\text{RAH}]/\Delta\delta_{\text{H-5'}}$ verses $[\text{RAH}]$ gave a linear fit (Fig. 31) confirming 1:1 stoichiometry for the RAH- β -CD complex. The slope of the plot is thus equal to $1/\Delta\delta_{\text{max}}$ and the intercept with the vertical axis to $\Delta\delta_{\text{max}}/K_a$, allowing the estimation of K_a to be 49 M^{-1} .

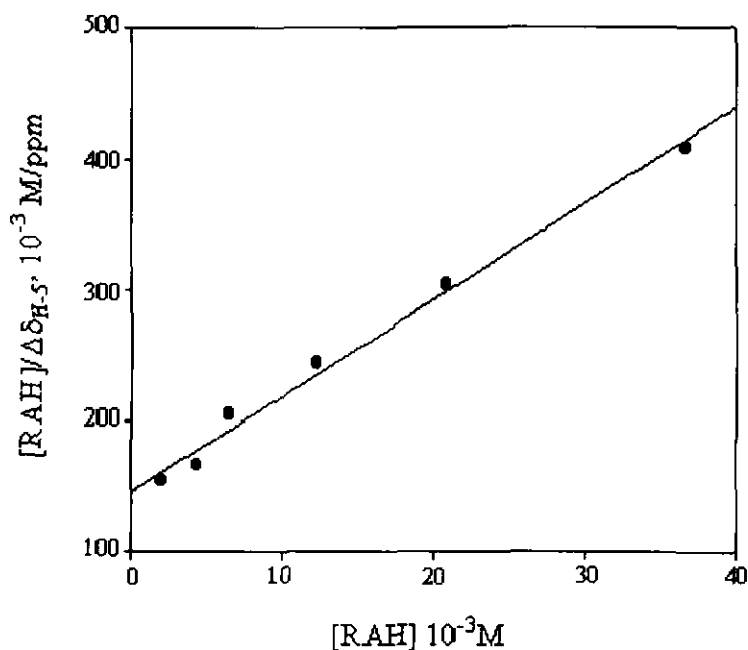


Fig. 31 Scott's plot of $[\text{RAH}]/\Delta\delta_{\text{H-5'}}$ verses $[\text{RAH}]$ showing 1:1 stoichiometry for RAH- β -CD complex.

¹H NMR Assignment of Roxatidine Acetate Hydrochloride

The assignment of all the signals in the ¹H NMR spectrum of pure RAH was made with the help of COSY spectrum (Fig. 29). The signals for RAH did not interfere with those of β-CD in the spectra of mixtures of RAH and β-CD. The assignment for all the proton signals of RAH is shown in Fig. 22. The aromatic protons were observed as a singlet at 6.980 (H-6), a multiplet for two protons at 7.023 (H-2, 4) and a triplet at 7.362 (H-3).

¹H NMR Chemical Shift Change Data of Roxatidine Acetate Hydrochloride

In the presence of β-CD, changes in the chemical shifts and shapes of the aromatic proton signals indicated the involvement of aromatic ring in complexation. The signal for H-6 exhibited highfield shift while those for H-2, 4 and H-3 displayed downfield shift changes. Aliphatic protons of RAH also exhibited chemical shift changes in the presence of β-CD but their chemical shift changes were not calculated because complexation involved only the aromatic ring of RAH. Fig.32 shows the changes in the aromatic region of the spectrum of RAH in the presence of β-CD compared to pure RAH.

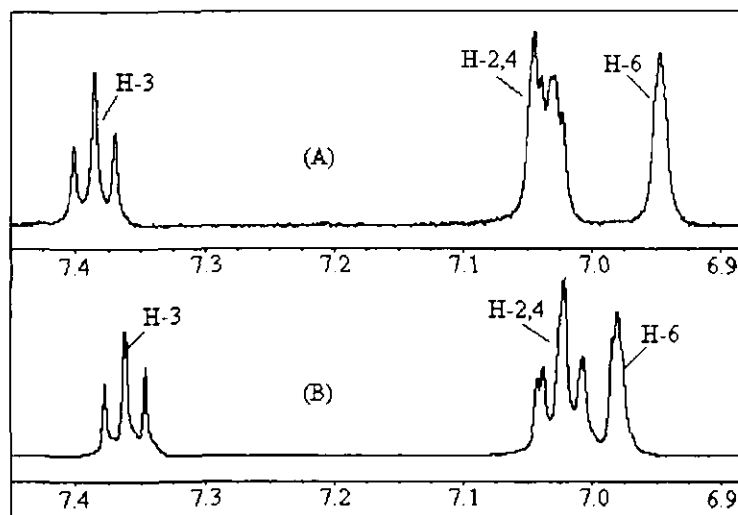


Fig. 32 Part of the ^1H NMR (500 MHz) spectra showing aromatic protons of RAH (A) 1:2.367 $[\beta\text{-CD}]/[\text{RAH}]$ mixture (B) pure RAH.

NOESY/ROESY Data of RAH- β -CD Mixture

ROESY spectrum was recorded for a mixture of RAH and β -CD which did not show any cross peak between aromatic protons of RAH and β -CD cavity protons. The NOESY spectrum (Fig. 33) of the same mixture was then recorded which proved helpful in the structural characterization of the complex. As expected the H-2, 4 protons of RAH displayed through space interaction with H-3' and H-6' of β -CD but not with H-5' proving the shallow penetration of the aromatic ring into the β -CD cavity from the wider side of the cavity. No aliphatic proton of RAH showed cross peak with β -CD cavity protons.

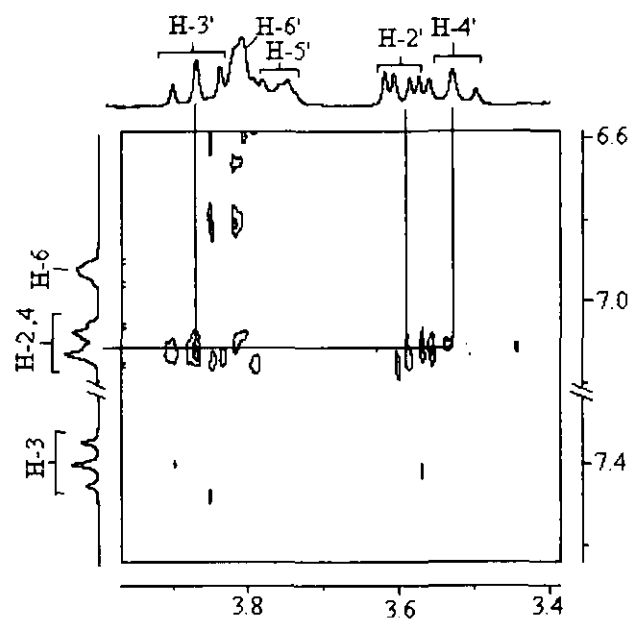


Fig. 33 A Partial NOESY spectrum (500 MHz) of a mixture of RAH and β -CD showing dipolar interactions between aromatic protons of RAH and β -CD cavity protons.

(Mixing time = 0.5 sec, Delay Time = 3.90 sec)

The proposed structure for the complex formed between RAH and β -CD in aqueous solution is shown in Fig. 34.

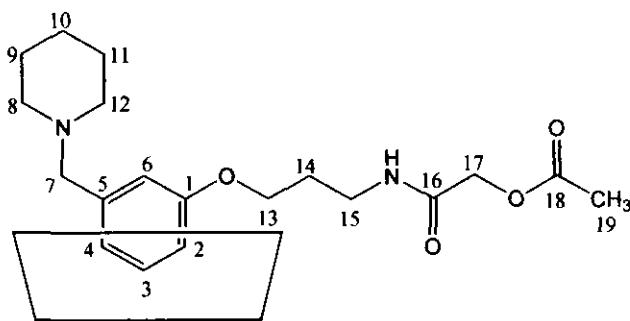
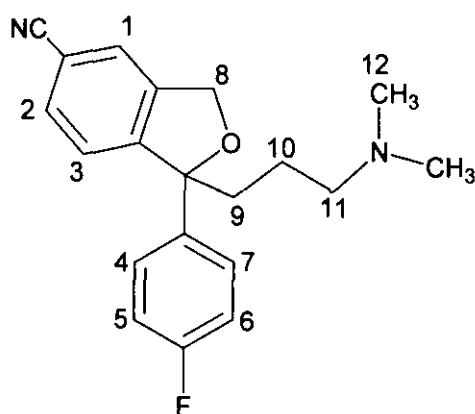


Fig. 34 Proposed structure for 1:1 RAH- β -CD complex.

*¹H NMR Spectroscopic Study of
Complexation of Citalopram
with β -Cyclodextrin*

Citalopram (CT), chemically known as (\pm) -1-[3-(dimethylamino)-propyl]-1-(4-fluorophenyl)-5-isobenzofurancarbonitrile, is a selective serotonin-reuptake inhibitor. Its ability to potentiate serotonergic activity in the central nervous system, via inhibition of the neuronal reuptake of serotonin, is thought to be responsible for its antidepressant action.²⁶³ It is marketed as racemic mixture though *S*-enantiomer of CT (escitalopram) has been found about 30-folds more potent than its *R*-counterpart.²⁶⁴



Citalopram

Experimental

All the ^1H NMR spectra (Figs. 35-40) were recorded on an Inova 500 MHz instrument in D_2O at 25 $^\circ\text{C}$. The chemical shift values, reported in δ (ppm), were calculated with reference to HDO peak at 4.780. To determine the stoichiometry of the complex, ^1H NMR shift data for CT and β -CD was obtained by keeping the overall concentration of the two components constant ($[\text{CT}] + [\beta\text{-CD}] = 10 \text{ mM}$) while the molar ratio ($r = [\text{CT}]$ or $[\beta\text{-CD}]/([\text{CT}] + [\beta\text{-CD}])$) was varied from 0 to 1. To determine the binding constant, ^1H NMR shift data for another set of six mixtures of CT and β -CD was obtained in which the concentration of CT was kept constant at 5 mM while that of β -CD was varied from 0.8 mM to 12 mM. COSY (Fig. 41) and ROESY (Fig. 42) spectra were recorded for a mixture of CT and β -CD. All the spectra consisted of one set of concentration dependent resonances for each proton or group of equivalent protons, indicating a fast reversible exchange between the free and complexed drug on the NMR time scale.

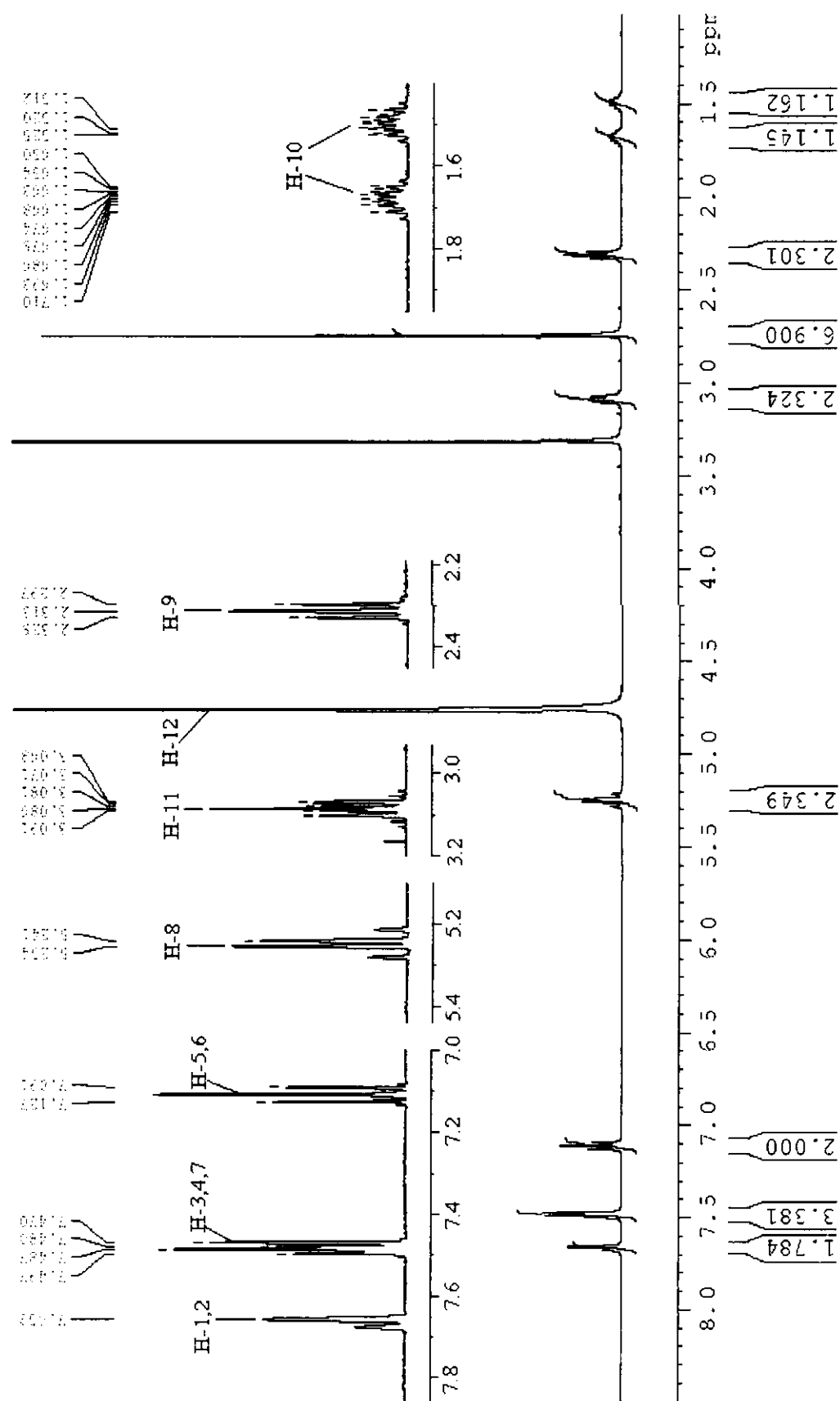


Fig. 35 ^1H NMR spectrum of pure citralopram.

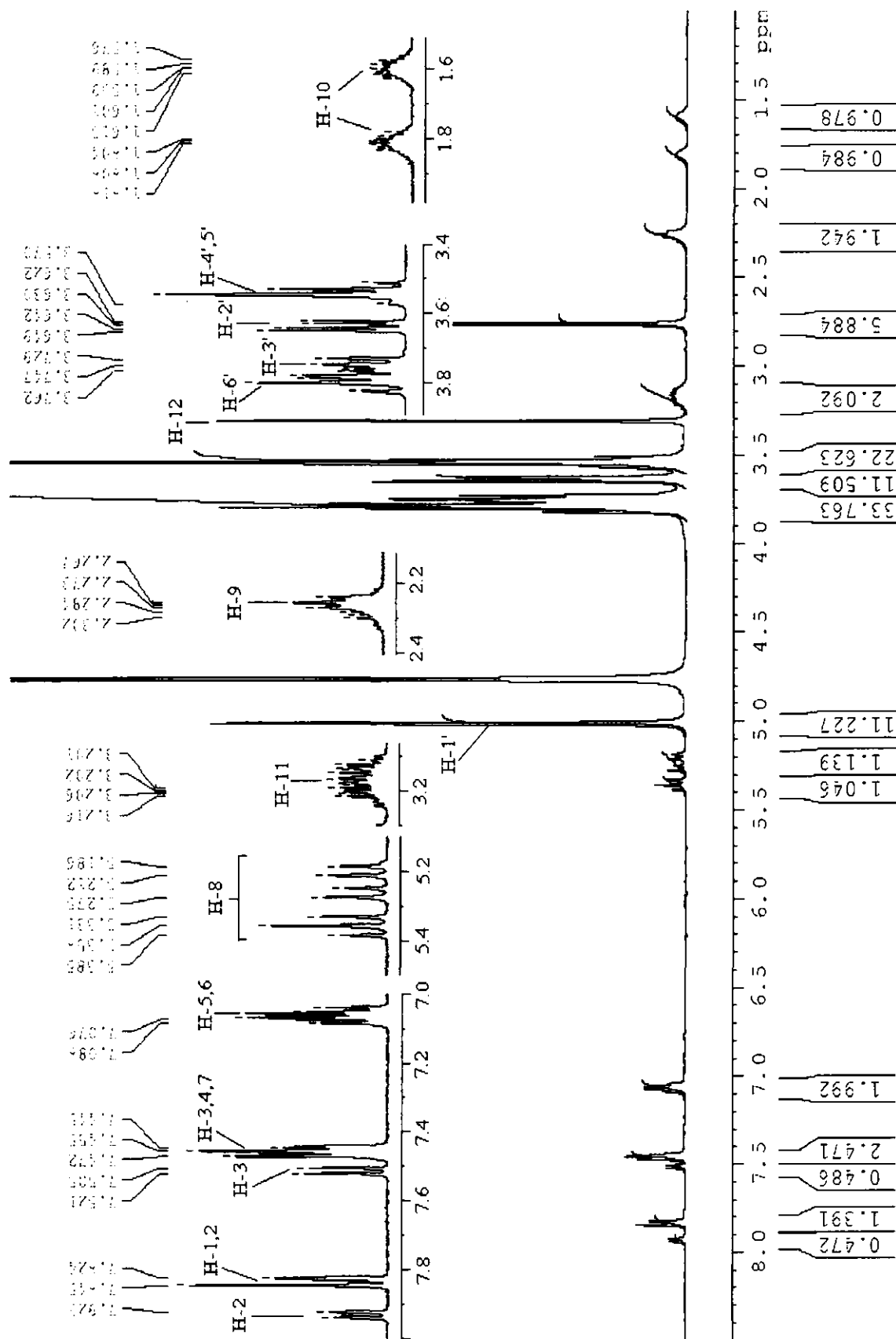


Fig. 36 ^1H NMR spectrum of a mixture of citralopram and β -CD having a molar ratio ($[\beta\text{-CD}]/[\text{CT}]$) equal to 1.609.

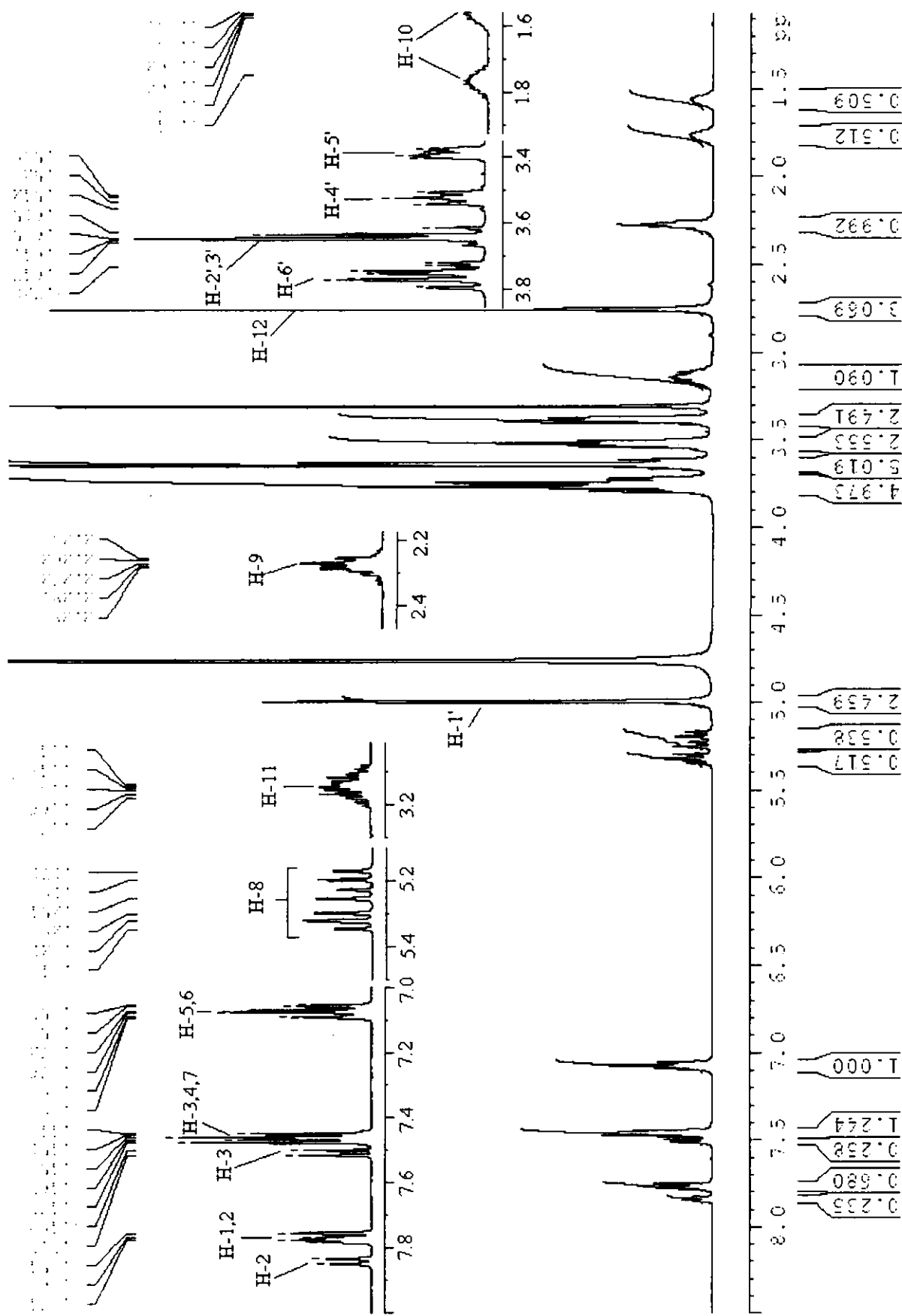


Fig. 37 ^1H NMR spectrum of a mixture of citralopram and β -CD having a molar ratio ($[\beta\text{-CD}]/[\text{CT}]$) equal to 1.245. 9

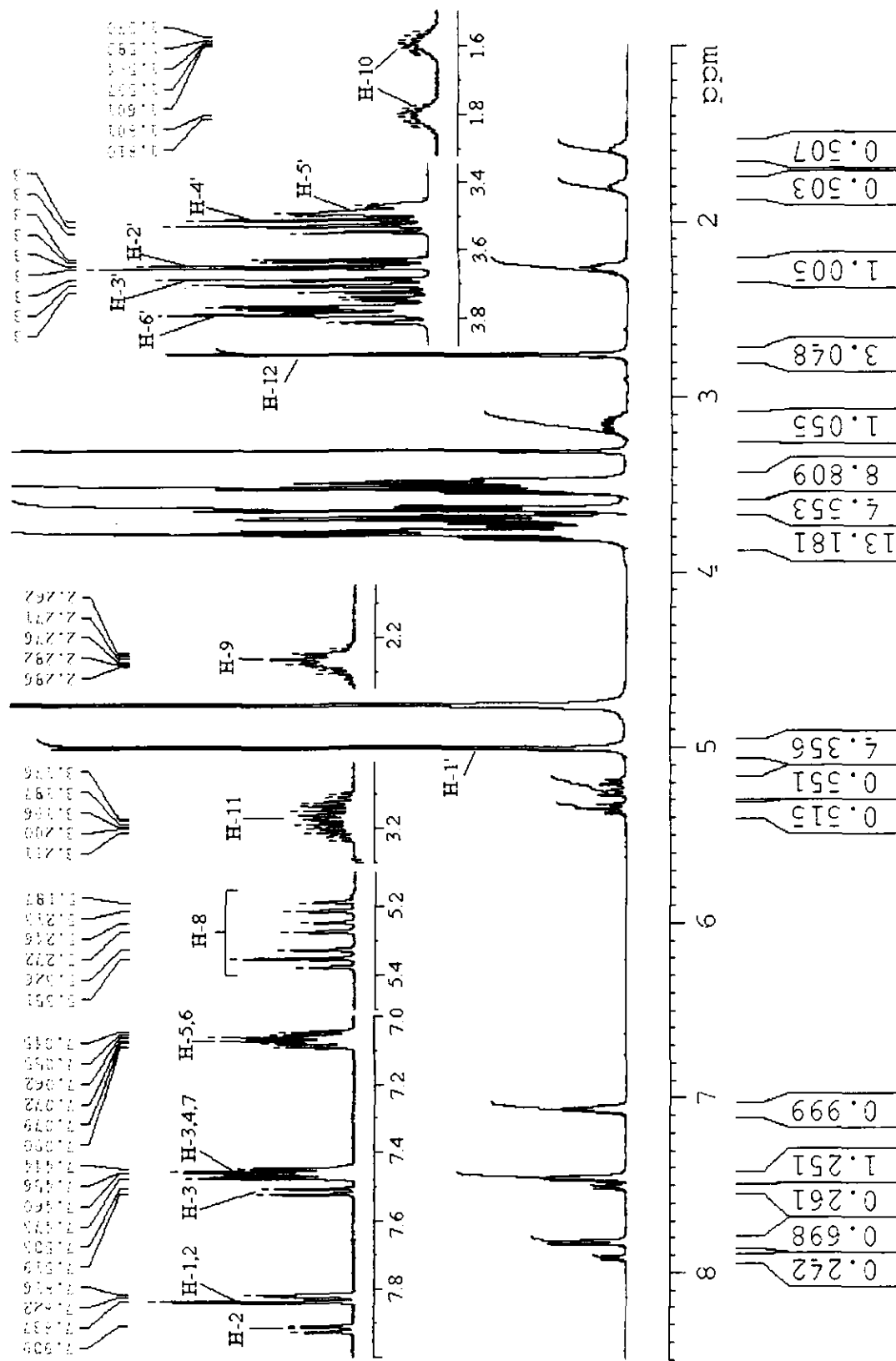


Fig. 38 ^1H NMR spectrum of a mixture of citralopram and β -CD having a molar ratio ($[\beta\text{-CD}]/[\text{CT}]$) equal to 0.702. 11

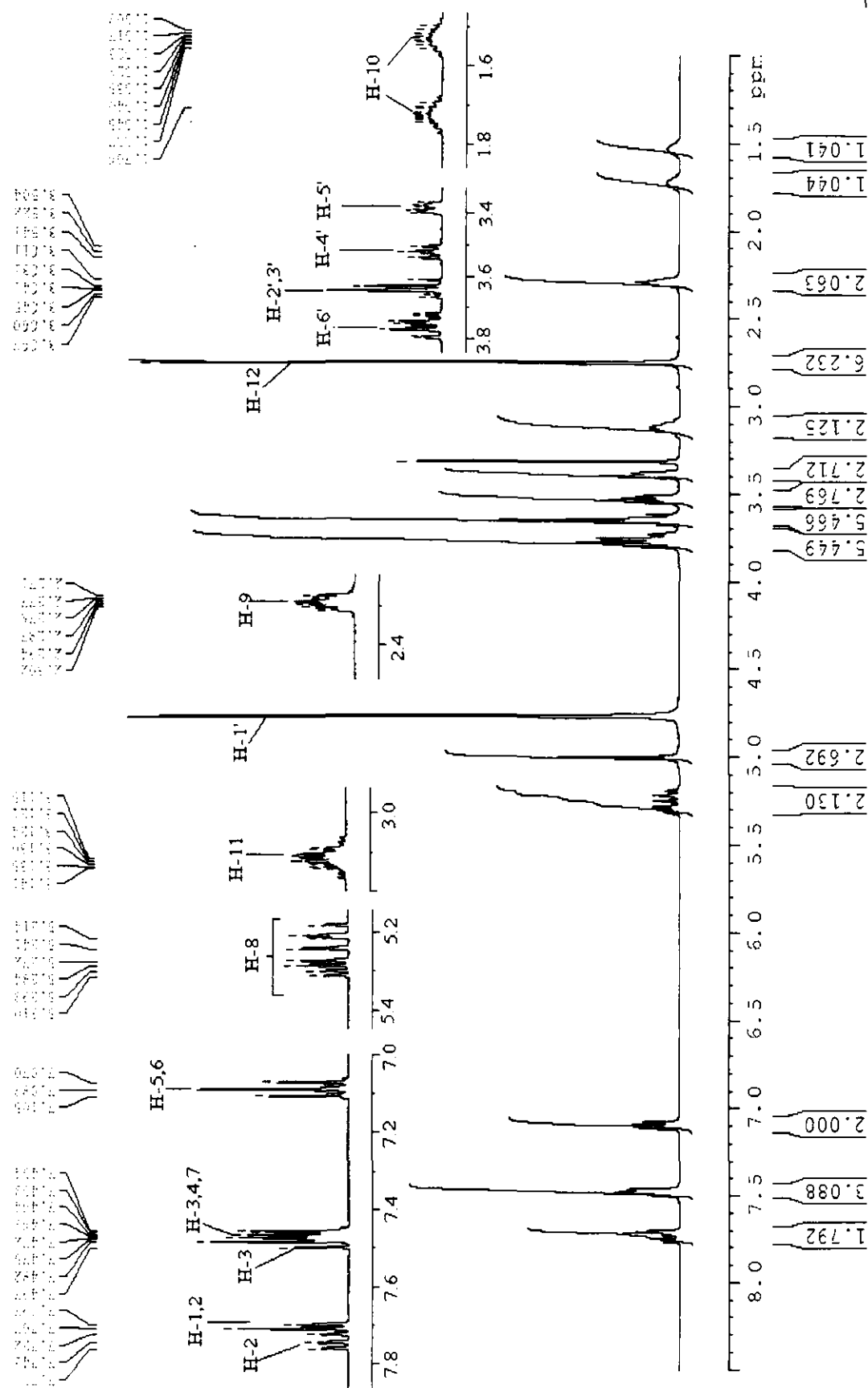


Fig. 39 ^1H NMR spectrum of a mixture of citralopram and β -CD having a molar ratio ($[\beta\text{-CD}]/[\text{CT}]$) equal to 0.384.

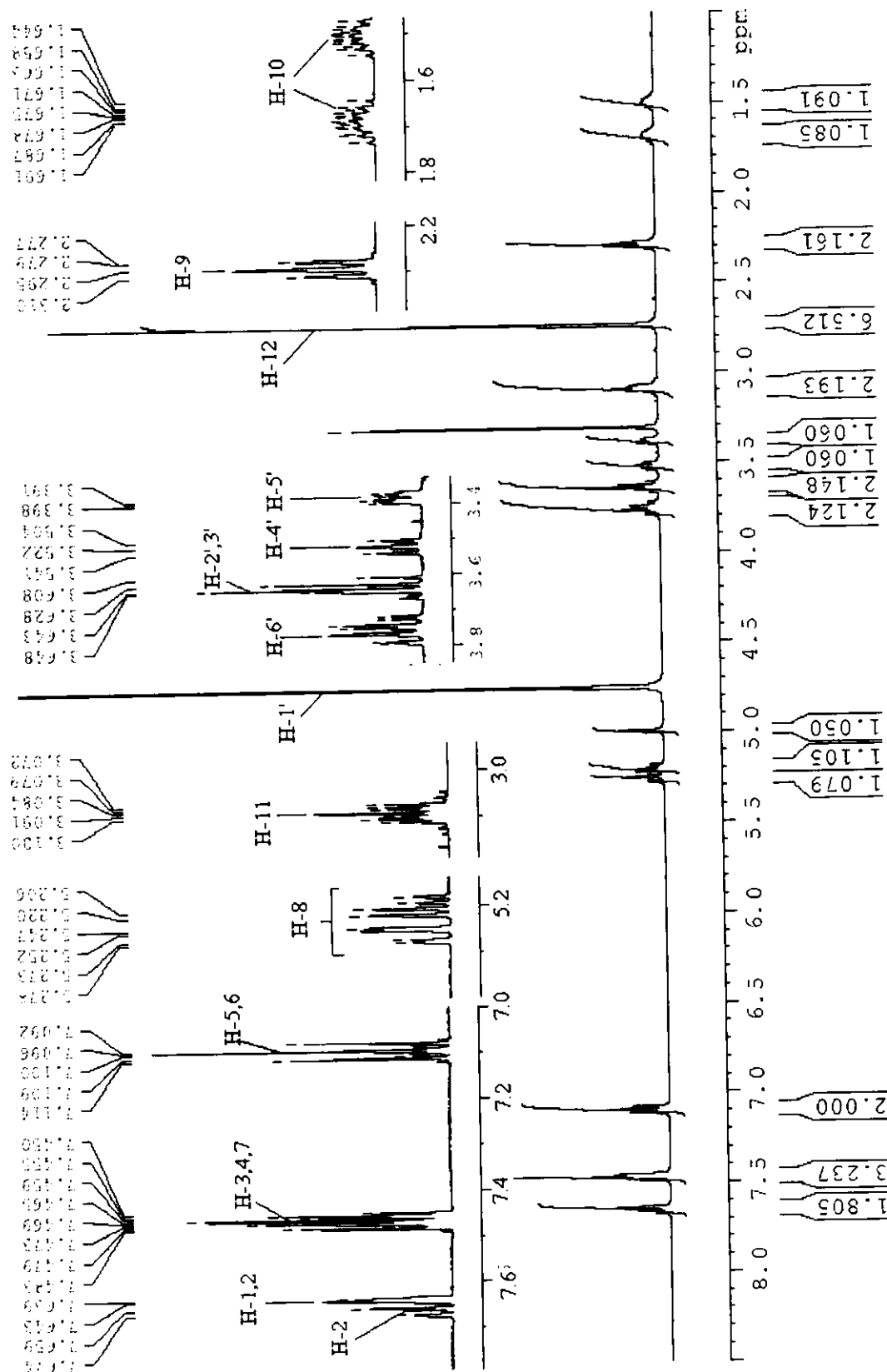


Fig. 40 ^1H NMR spectrum of a mixture of citralopram and β -CD having a molar ratio ($[\beta\text{-CD}]/[\text{CT}]$) equal to 0.150. 112

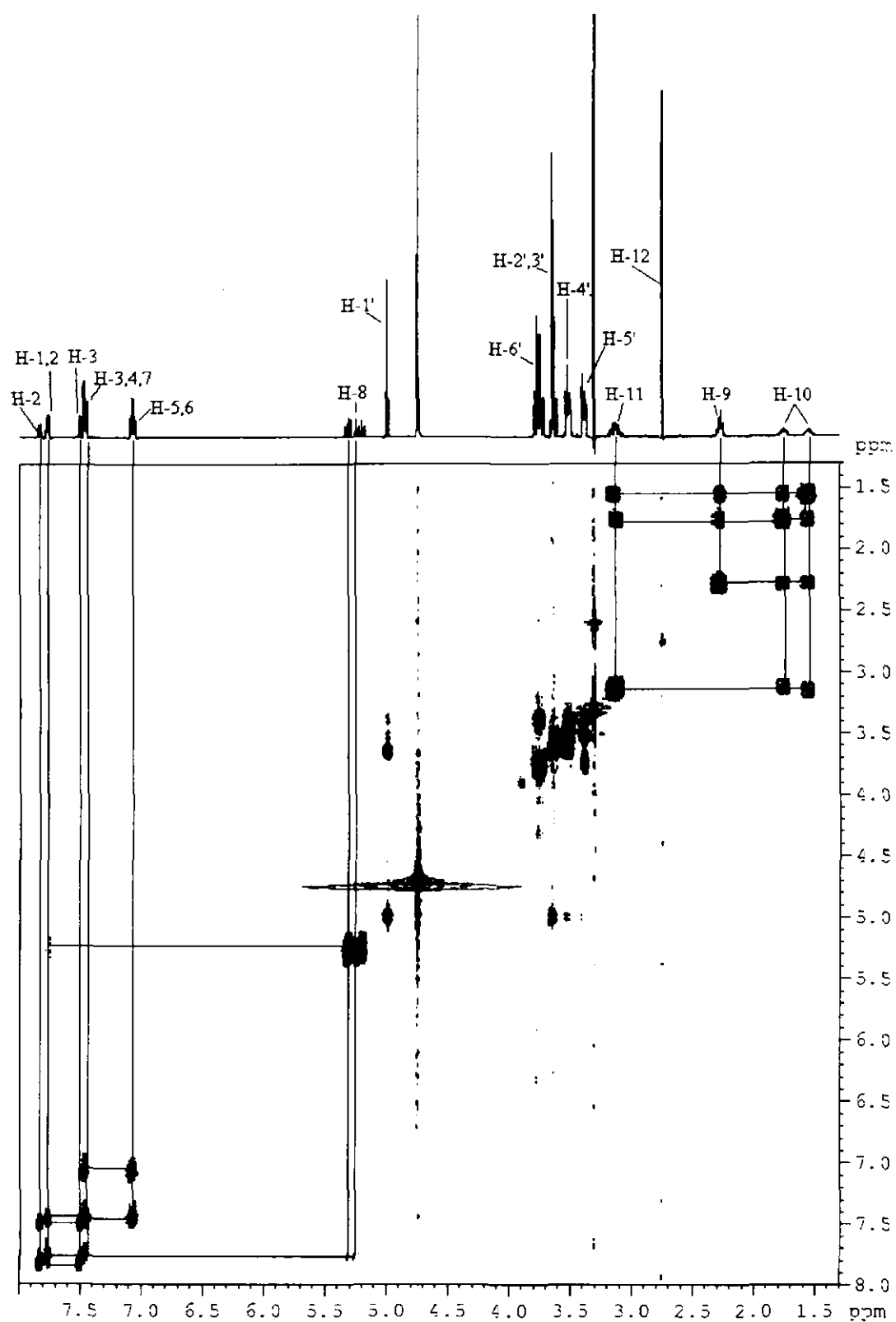


Fig. 41 2D COSY spectrum for a mixture of CT and β -CD.

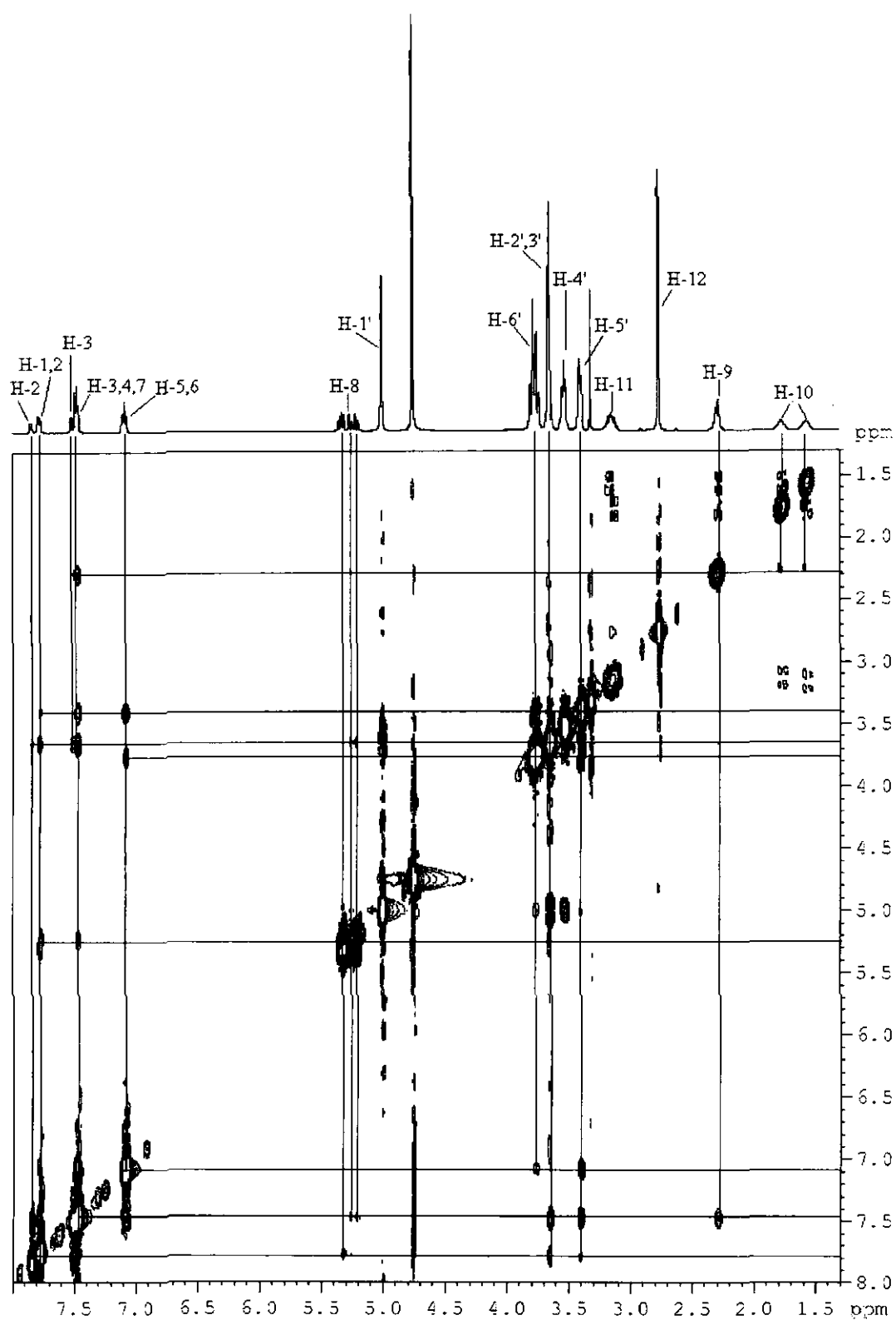


Fig. 42 2D ROESY spectrum for a mixture of CT and β -CD.

Result and Discussion

¹H NMR Chemical Shift Change Data of β -CD

The assignment of β -CD cavity protons was made with the help of COSY spectral data (Fig. 41). The signal for H-1' appears separate from rest of the β -CD protons at 4.995 and can easily be identified. It shows cross peak with H-2' and thus all β -CD protons are easily recognized by studying the cross peaks between various protons in the COSY spectrum (Fig. 43).

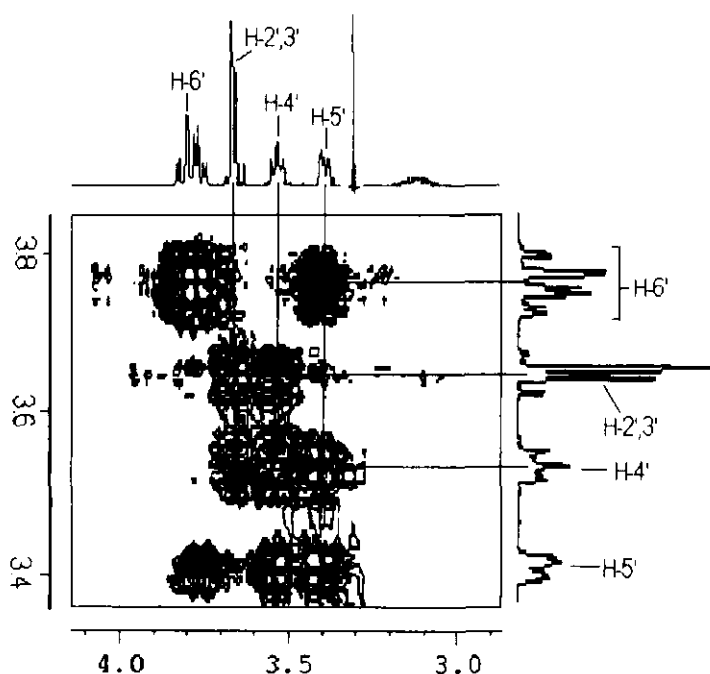


Fig. 43 Part of COSY spectrum (500 MHz) of a mixture of CT and β -CD showing β -CD signals.

The β -CD cavity protons, namely H-3' and H-5', displayed significant highfield shift changes in the presence of CT which increased with the increasing concentration of CT

while other β -CD protons exhibited negligible shifts. Fig. 44 shows changes in the chemical shifts of β -CD protons in the presence of CT. The chemical shift changes for all the β -CD protons in the presence of CT are given in Table 11. The highfield shift changes in the β -CD cavity protons can only be attributed to the penetration of the guest, most probably aromatic ring, into the β -CD cavity resulting in the formation of β -CD-CT inclusion complex/es, in analogy to previous studies.^{233, 237, 261, 262}

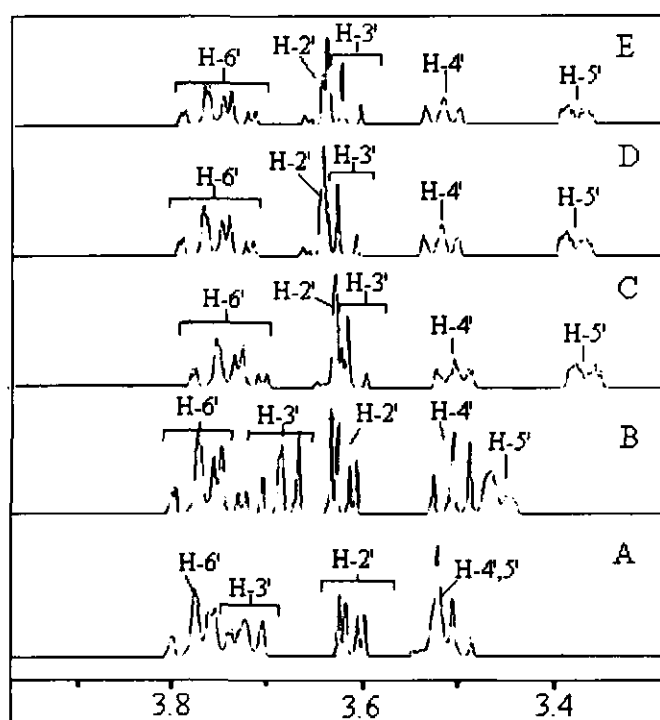


Fig. 44 Part of ^1H NMR spectra (500 MHz) showing β -CD proton signals of mixtures of β -CD and CT having molar ratios ($[\beta\text{-CD}]/[\text{CT}]$) (A) 1.609 (B) 1.245 (C) 0.702 (D) 0.384 (E) 0.150. (Temperature = 25 $^\circ\text{C}$, Solvent = D_2O)

Table 11 ^1H NMR chemical shift change ($\Delta\delta$) data for β -CD protons, in the presence of CT.

$[\beta\text{-CD}]/[\text{CT}]$	H-1'	H-2'	H-3'	H-4'	H-5'	H-6'
1.609	0.002	0.043	-0.150	0.009	-0.213	-0.027
1.245	0.008	0.048	-0.198	0.000	-0.313	-0.043
0.702	0.016	0.048	-0.262	0.006	-0.405	-0.062
0.384	0.018	0.049	-0.266	0.007	-0.410	-0.063
0.150	0.018	0.053	-0.269	0.007	-0.413	-0.063

Negative values indicate upfield shift

(Temperature = 25 °C, Solvent = D_2O)

^1H NMR Assignment of Citalopram

An unambiguous ^1H NMR assignment of the guest, especially aromatic protons, is important for the study of β -CD inclusion complexes. The assignment of all the CT protons was made with the help of COSY (Fig. 41) and ROESY (Fig. 42) spectral data of CT in the presence of β -CD because some of the protons, which appeared completely merged in the spectrum of pure CT, separated in the presence of β -CD helping in assignment. The ^1H NMR spectral data of CT in the absence as well as in the presence of β -CD is given in Table 12. The signals for H-9 and H-11 were observed as triplets at 2.313 ($J=8.0$ Hz) and 3.086 ($J = 7.5$ Hz) in the spectrum of pure CT but appeared as multiplets in the presence of β -CD due to splitting of signals. The signals for H-10 appeared as a pair of multiplets in the presence as well as in the absence of β -CD. The assignment of these signals is supported by the cross peaks between H-9/H-10 and H-10/H-11 and no H-9/H-11 cross peaks in the COSY spectrum. The signal for H-12 was observed as a singlet in the absence as well as presence of β -CD. A pair of doublets at 5.228 ($J=.15$ Hz) and 5.265 ($J=17.4$ Hz), each integrating for one proton, in the spectrum of pure CT was ascribable to only H-8. Each of these doublets splitted into a pair of

doublets in the presence of β -CD. The aromatic protons were observed as a triplet at 7.106 ($J=10.5$, 2H) and multiplets centered at 7.483 (3H) and 7.654 (2H). As the fluorine-containing ring (A) protons are expected to appear relatively highfield compared to cyano-containing ring (B), the triplet at 7.106 could only be ascribed to ring A protons while the signal at 7.654 could be assigned to ring B protons. The protons H-5, 6 should interact with H-4, 7 and adjacent fluorine atom and appear as triplet. The signal at 7.106 was, therefore, ascribed to H-5, 6. The signal at 7.654 was assigned to H-1, 2 of ring B because protons ortho to cyano group should appear downfield. The multiplet at 7.483 can only be due to H-4, 7 (A) and H-3 (B). These assignments are well supported by the COSY and ROESY spectral data. The H-4, 7, being in close proximity to H-8 and H-9, showed cross connection peaks with these protons in the ROESY spectrum. Similarly, cross peaks between H-1 and H-8 were observed in the ROESY spectrum. In the presence of β -CD, the signals for H-2 and H-3 of one enantiomer separated completely while those for other enantiomer remain merged with H-1 and H-4, 7 signals, respectively. That the separated signals belong to same enantiomer is evidenced by the presence of cross peaks between these signals in the COSY spectrum (Fig. 45).

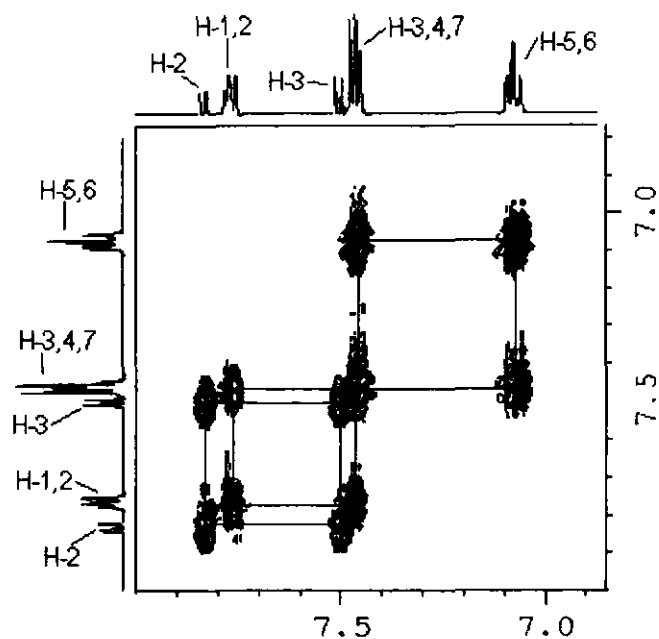


Fig. 45 Part of the COSY Spectrum (500 MHz) of a mixture of CT and β -CD showing cross peaks of aromatic protons of CT.

^1H NMR Chemical Shift Change Data of Citalopram

All the CT protons exhibited significant chemical shift changes in the presence of β -CD which increased with the increase in concentration of the β -CD. The signals for the ring A protons, H-9 and one of the H-8, for one enantiomer, displayed highfield chemical shift changes while rest of the proton resonances moved downfield. The magnitude of the chemical shift changes for ring B protons was much larger compared to protons of the ring A. Moreover, all the proton signals of the CT splitted in the presence of β -CD because of

the chiral discrimination by the β -CD between the two enantiomers. The separation of the enantiomeric signals was highest for H-2, 3 and H-8 protons. The chemical shift change data for all the CT protons in the presence of β -CD is given in Table 12 while Fig. 46 shows the shift changes and splitting of the CT proton resonances in the presence of β -CD compared to pure CT.

Table 12 ^1H NMR (500 MHz) chemical shift change ($\Delta\delta$, ppm) data for CT and $[\beta\text{-CD}]/[\text{CT}]$ mixtures for all the protons of CT in the presence of β -CD in D_2O . (Temperature = 25 $^\circ\text{C}$)

Proton	[CT]	$[\beta\text{-CD}]/[\text{CT}]$ =1.609	$[\beta\text{-CD}]/[\text{CT}]$ =1.245	$[\beta\text{-CD}]/[\text{CT}]$ =0.702	$[\beta\text{-CD}]/[\text{CT}]$ =0.384	$[\beta\text{-CD}]/[\text{CT}]$ =0.150
H-10	1.500	0.099	0.085	0.058	0.048	0.010
	1.680	0.128	0.125	0.089	0.044	0.009
H-9	2.313	-0.054	-0.051	-0.041	-0.026	-0.018
H-12	2.744	0.020	0.019	0.011	0.003	-0.004
H-11	3.086	0.085	0.080	0.057	0.028	0.000
H-8	5.228 [#]	-0.029	-0.023	-0.044	-0.032	-0.039
		0.038	0.031	0.010	-0.003	-0.015
	5.265 [#]	0.079	0.034	0.040	0.013	-0.016
		0.106	0.073	0.069	0.039	0.010
H-5, 6	7.106	-0.040	-0.039	-0.032	-0.018	-0.005
H-4, 7	7.483	-0.028	-0.025	-0.022	-0.016	-0.010
H-3	7.483 [#]	-0.028	-0.025	-0.022	-0.016	-0.010
		0.030	0.028	0.024	0.006	0.001
H-1	7.654	0.180	0.172	0.118	0.055	-0.013
H-2	7.654 [#]	0.180	0.172	0.118	0.055	-0.013
		0.279	0.268	0.184	0.097	0.012

Negative values indicate highfield shift

[#]The signal splitted in the presence of β -CD

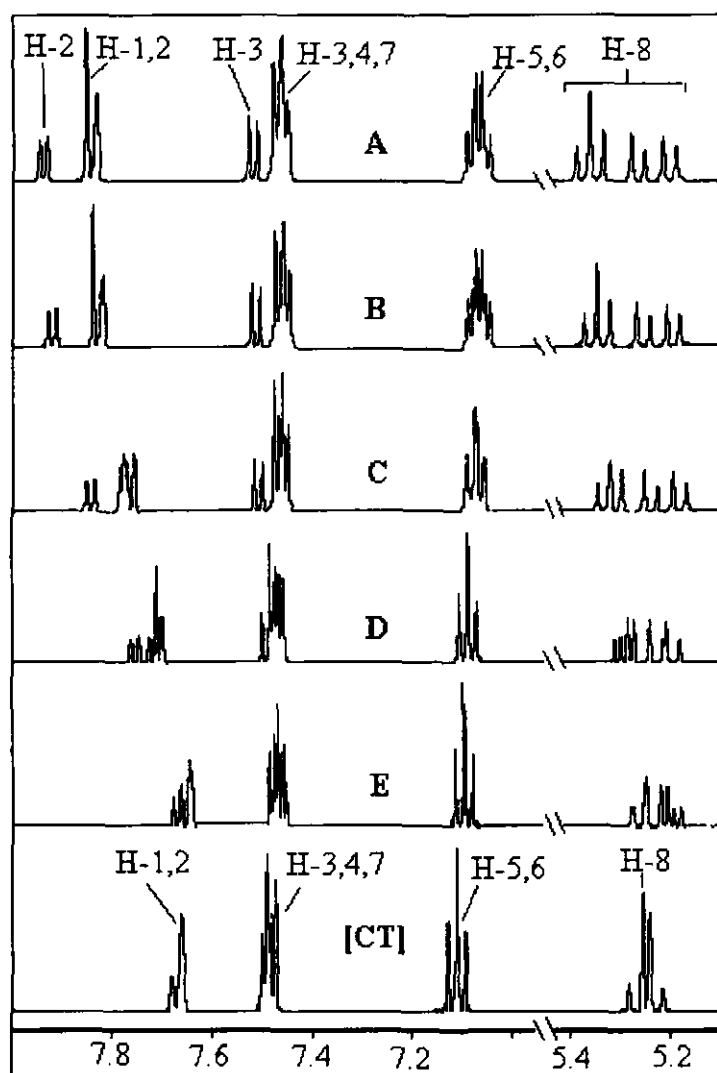
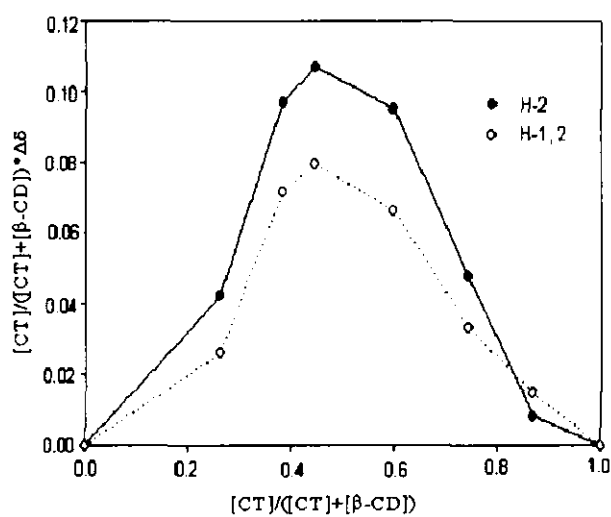


Fig. 46 Parts of ^1H NMR spectra (500 MHz) of mixtures of β -CD and CT showing CT proton signals in comparison to pure CT. ($[\beta\text{-CD}]/[\text{CT}]$ (A) 1.609 (B) 1.245 (C) 0.702 (D) 0.384 (E) 0.150). (Temperature = 25 $^\circ\text{C}$, Solvent = D_2O)

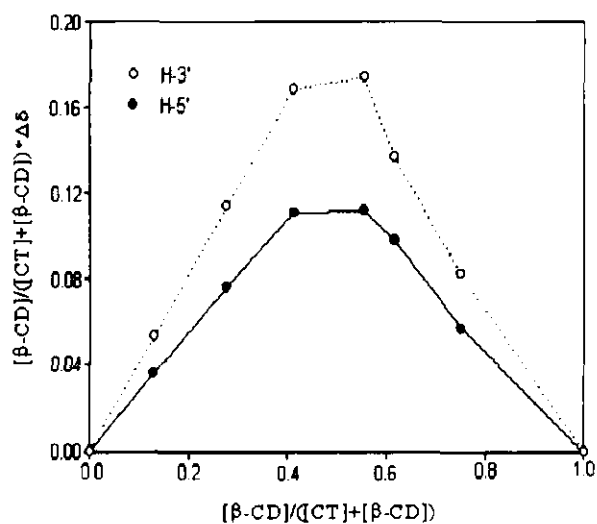
Stoichiometry of the β -CD-CT Complex

To establish the stoichiometry of the β -CD-CT complex, the continuous variation method²⁴⁹ (Job's) was used to follow the changes in the chemical shifts of H-1, 2 of CT

(Fig. 47 A) and H-3' and H-5' of β -CD (Fig. 47 B). Although the shapes of the curves are not highly symmetrical, the maxima corresponds to a molar ratio (r) of 0.42 indicating that there is one molecule of CT for each β -CD molecule in the complex i.e. 1:1 stoichiometry of the β -CD- CT complex/es.



A



B

Fig. 47 Job's plot for the β -CD/CT complex (A) plot of data corresponding to H-2 and H-1,2 protons of CT (B) plot for data corresponding to H-3' and H-5' protons of β -CD.

The overall stability constant (K_a) of the complex/es was determined using Scott's modification²⁵⁶ of Benesi-Hildebrand equation.²⁵¹

$$[H]/\Delta\delta_{\text{obs}} = [H]/\Delta\delta_{\text{max}} + \Delta\delta_{\text{max}}/K_a$$

The plots of $\Delta\delta_{\text{H-2}}$ or $\Delta\delta_{\text{H-1, 2}}$ against $[\beta\text{-CD}]$ in the form of $[\beta\text{-CD}]/\Delta\delta_{\text{obs}}$ vs $[\beta\text{-CD}]$ gave excellent linear fits (Fig. 48) confirming 1:1 $\beta\text{-CD-CT}$ complexation. The slope of the plot is thus equal to $1/\Delta\delta_{\text{max}}$ and the intercept with the vertical axis to $\Delta\delta_{\text{max}}/K_a$, allowing the estimation of K_a to be 436 M^{-1} which is the mean value of the K_a determined from two plots.

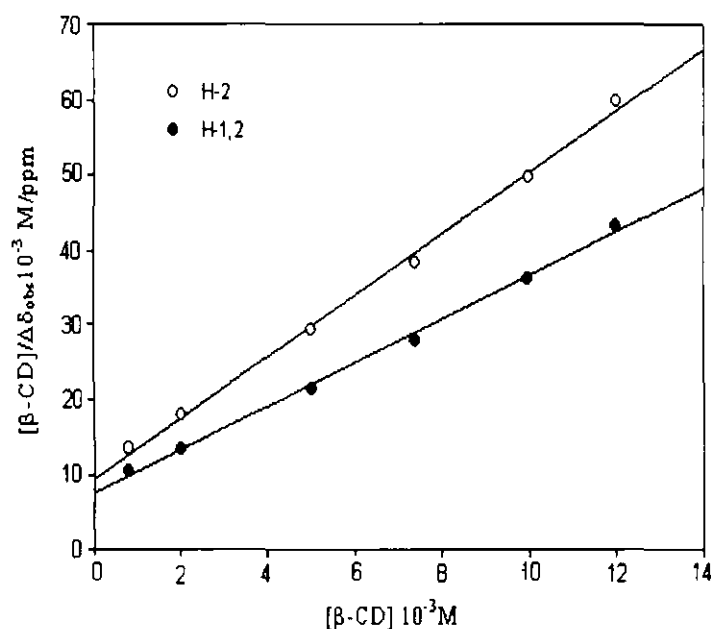


Fig. 48 Scott's plot of $[\beta\text{-CD}]/\Delta\delta_{\text{obs}}$ verses $[\beta\text{-CD}]$ showing

1:1 stoichiometry for $\beta\text{-CD-CT}$ complex.

ROESY Spectral Data of β -CD-CT Mixture

There are two aromatic rings present in the CT and NOE studies were carried out under rotating frame conditions (ROESY)²³² to establish beyond doubt the involvement of these rings in the complexation and to determine the actual structure of the complex. ROESY spectral data of a mixture of CT and β -CD confirmed that both the aromatic rings are incorporated into the β -CD cavity (Fig. 49). Moreover, the mode and depth of penetration of the two aromatic rings could also be established with the help of ROESY spectral data. An expansion of a part of the ROESY spectrum showing interactions of various protons of CT with the β -CD cavity protons is shown in Fig. 49.

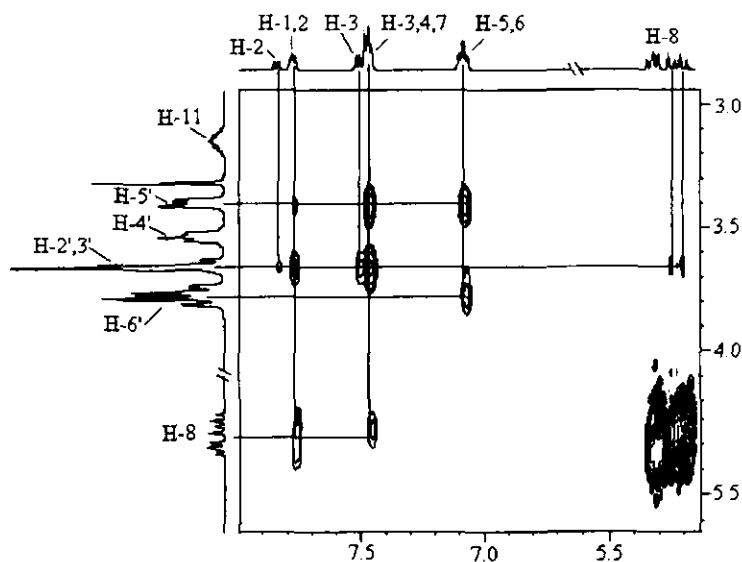
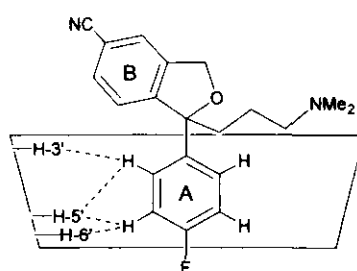


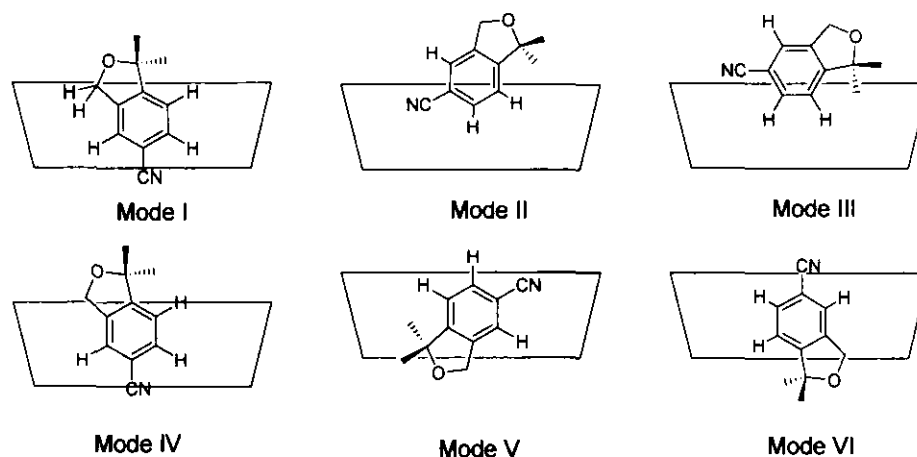
Fig. 49 A Partial ROESY (500 MHz) spectrum of a mixture of CT and β -CD showing dipolar interactions between CT and β -CD cavity protons.
(Mixing time = 0.2 sec, Delay Time = 3.35 sec)

Cross connection peaks between both the aromatic rings protons with β -CD cavity protons were observed. The signal for H-5, 6 of F-containing ring (A) displayed cross peaks with H-5' and H-6' of β -CD, both positioned near the narrower rim of the cavity, while H-4, 7 exhibited cross peaks with H-3' (located near wider rim) and H-5'.²³⁹ These results can only be explained by the mode of penetration of F-containing ring (A) as depicted below.



Also, all the CN-containing ring (B) proton signals displayed cross peaks with β -CD cavity protons. The signals for H-2 and H-3, for the two enantiomers, completely separated in the presence of β -CD. The H-2 and H-3 of one enantiomer (the signals for these protons for the other enantiomer appeared merged with other signals) exhibited cross peaks with only H-3' of β -CD. Moreover, the H-2/H-3' interaction was very weak compared to H-3/H-3'. Of the various possible modes of penetration of aromatic ring B into the CD cavity (**Modes I-VI**), only the modes in which H-2 lies along the axis of the CD-cavity (**Mode II, V**) can explain the interaction with only H-3' of β -CD and the difference in the intensity of peaks.²³⁸ Mode V can be ruled out due to steric factors²³⁸ thus **Mode II** seems the most probable structure of one β -CD-CT complex. The interaction between nitrile group and cavity protons may be the reason for more downfield shift changes in the H-2, 3 signals of citalopram. But this mode can not explain

the interaction between H-8 of CT and H-3' of β -CD which means that the two enantiomers have different modes of penetration in the complexes involving ring B and the only plausible structure for the other β -CD-CT complex can be as shown in **Mode I**.



In conclusion, racemic CT forms four 1:1 inclusion complexes with β -CD in aqueous solution by the penetration of aromatic ring into the β -CD cavity. The mode of penetration of the F-containing ring of *R*- and *S*-enantiomers seems identical and the mechanism of chiral differentiation by the β -CD between the two enantiomers of CT appears to be due to the difference in the mode of entry of the CN-containing ring (B) of the two enantiomers into the β -CD cavity. However, it is not possible to say with certainty which of the two structures belong to *R*-enantiomer and which to *S*-enantiomer. Fig. 50 shows all the 1:1 β -CD-CT complexes present in solution.

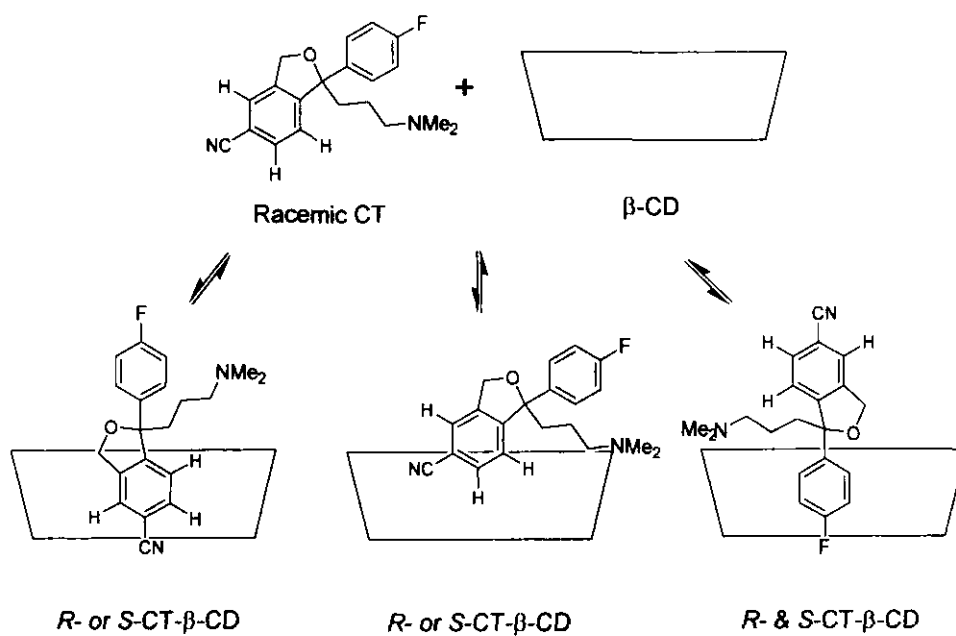
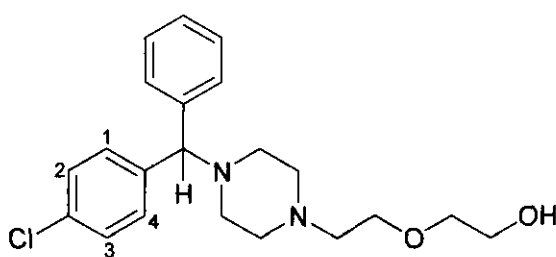


Fig. 50 Schematic representation of all the possible 1:1 inclusion complexes formed between CT and β-CD in D₂O.

*¹H NMR Spectroscopic Study of
Complexation of Hydroxyzine
Hydrochloride with β -Cyclodextrin*

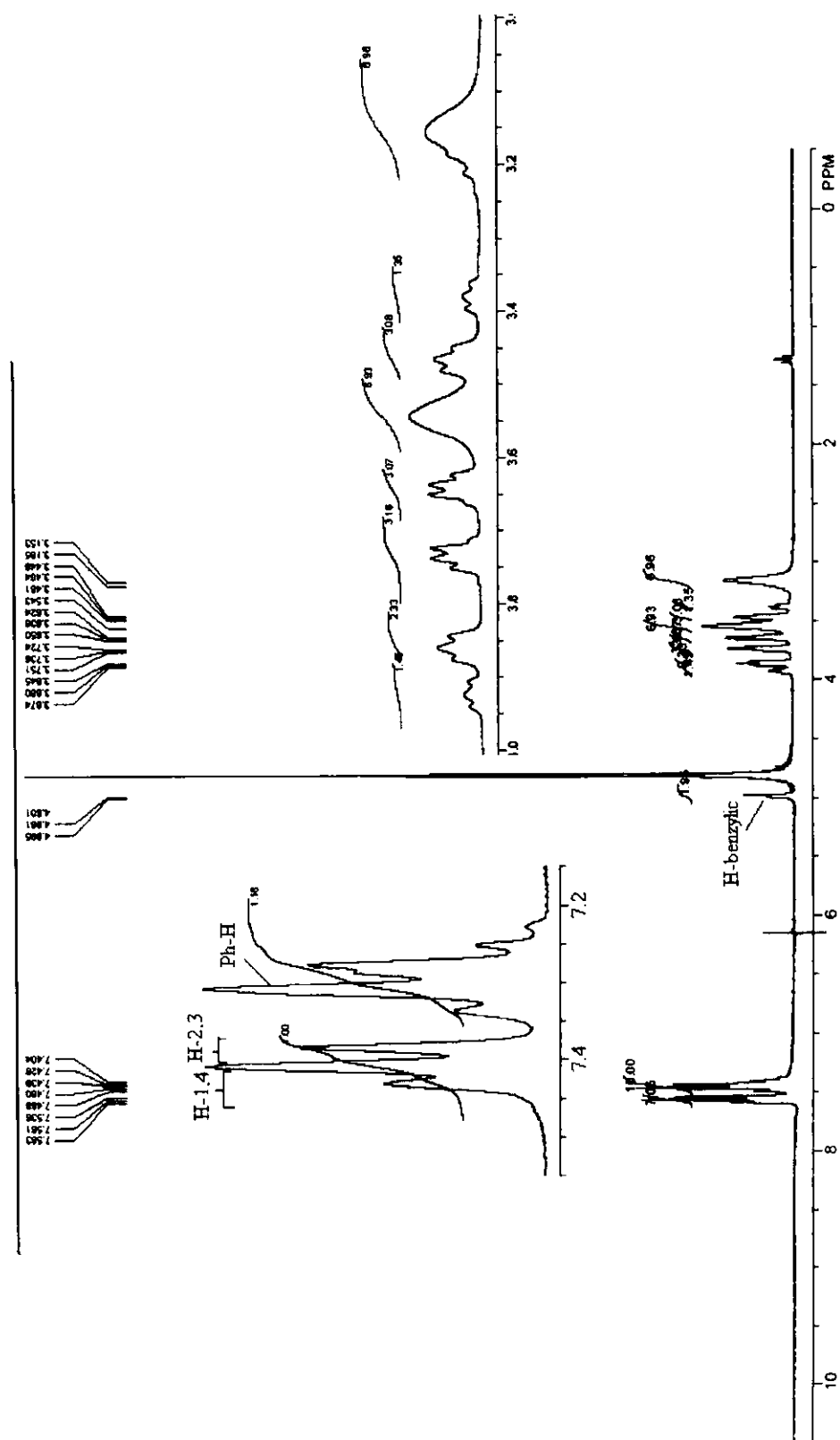
Hydroxyzine hydrochloride,²⁶⁵ (HYZ) chemically known as 2-(2-{4-[(RS)-(4-chlorophenyl)phenylmethyl]piperazine-1-yl}ethoxy)ethanol dihydrochloride, a piperazine derivative, belongs to antihistamine family. In addition to its antihistaminic effects, HYZ possesses anticholinergic, sedative, antispasmodic, tranquilizing, bronchodilative and antiemetic activities.²⁶⁶



Hydroxyzine

Experimental

All the experiments were performed on a Varian 300 MHz Unity instrument at room temperature for pure HYZ (Fig. 51), pure β -CD and for mixtures of HYZ and β -CD (Figs. 52-56). The ^1H NMR chemical shift data for β -CD cavity protons was obtained, by keeping the concentration of β -CD constant at 10 mM while varying the concentration of HYZ from 3.47 to 10 mM, to determine the stoichiometry and binding constant of the complex/es. The ROESY and COSY spectra of a mixture of HYZ and β -CD were recorded on a Bruker DRX 600 (600 MHz) instrument. All the spectra consisted of one set of concentration dependent resonances, for each proton or group of equivalent protons, indicating that the reversible exchange between free and complexed drug is rapid on the NMR time scale. The water signal at 4.800 ppm was used as internal reference throughout this work.



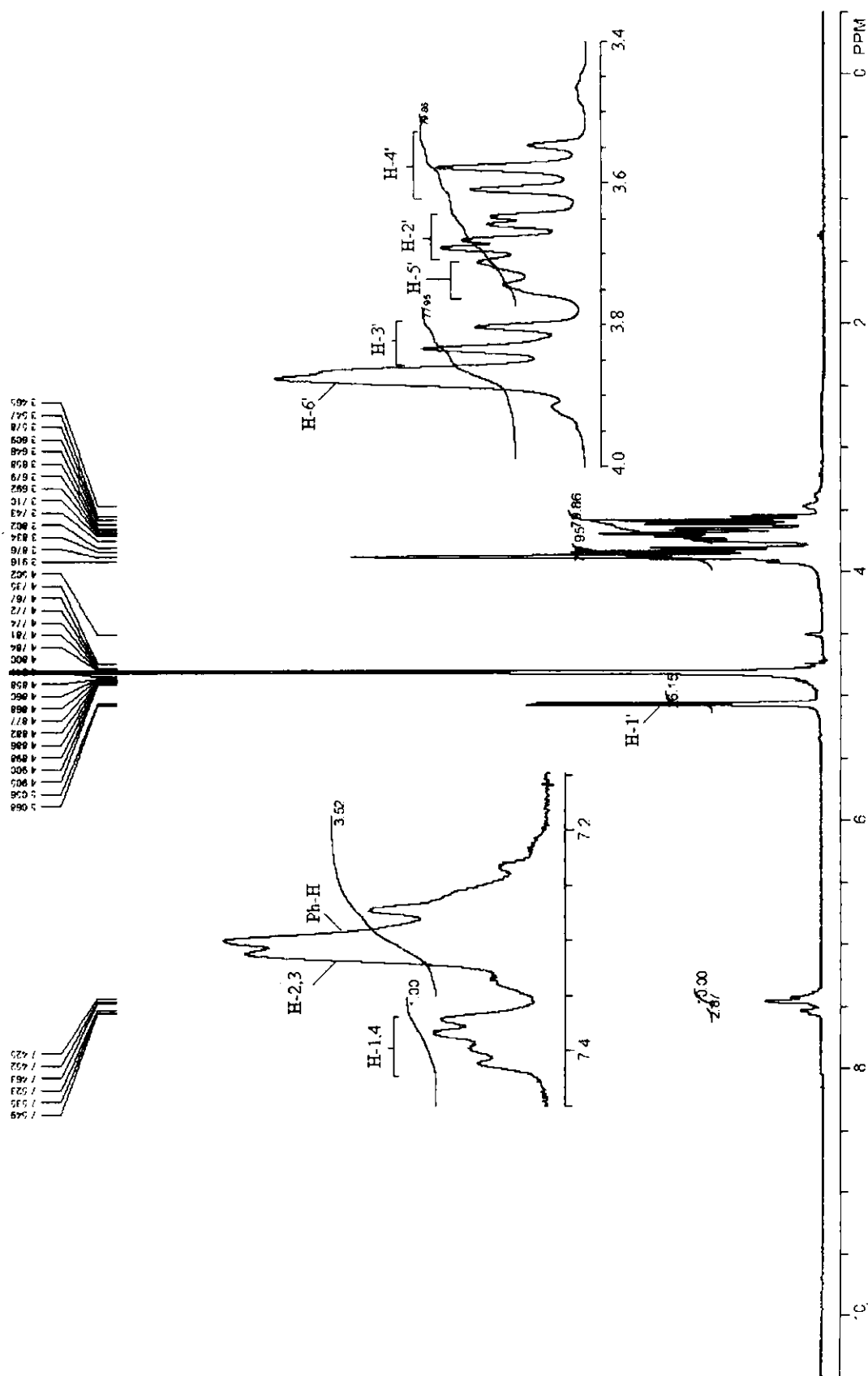


Fig. 53 ^1H NMR spectrum of a mixture of hydroxyzine hydrochloride and β -CD having a molar ratio $[\beta\text{-CD}]/[\text{HYZ}]$ equal to 2.50.

Fig. 54 ^1H NMR spectrum of a mixture of hydroxyzine hydrochloride and β -CD having a molar ratio $([\beta\text{-CD}]/[\text{HYZ}])$ equal to 2.18.

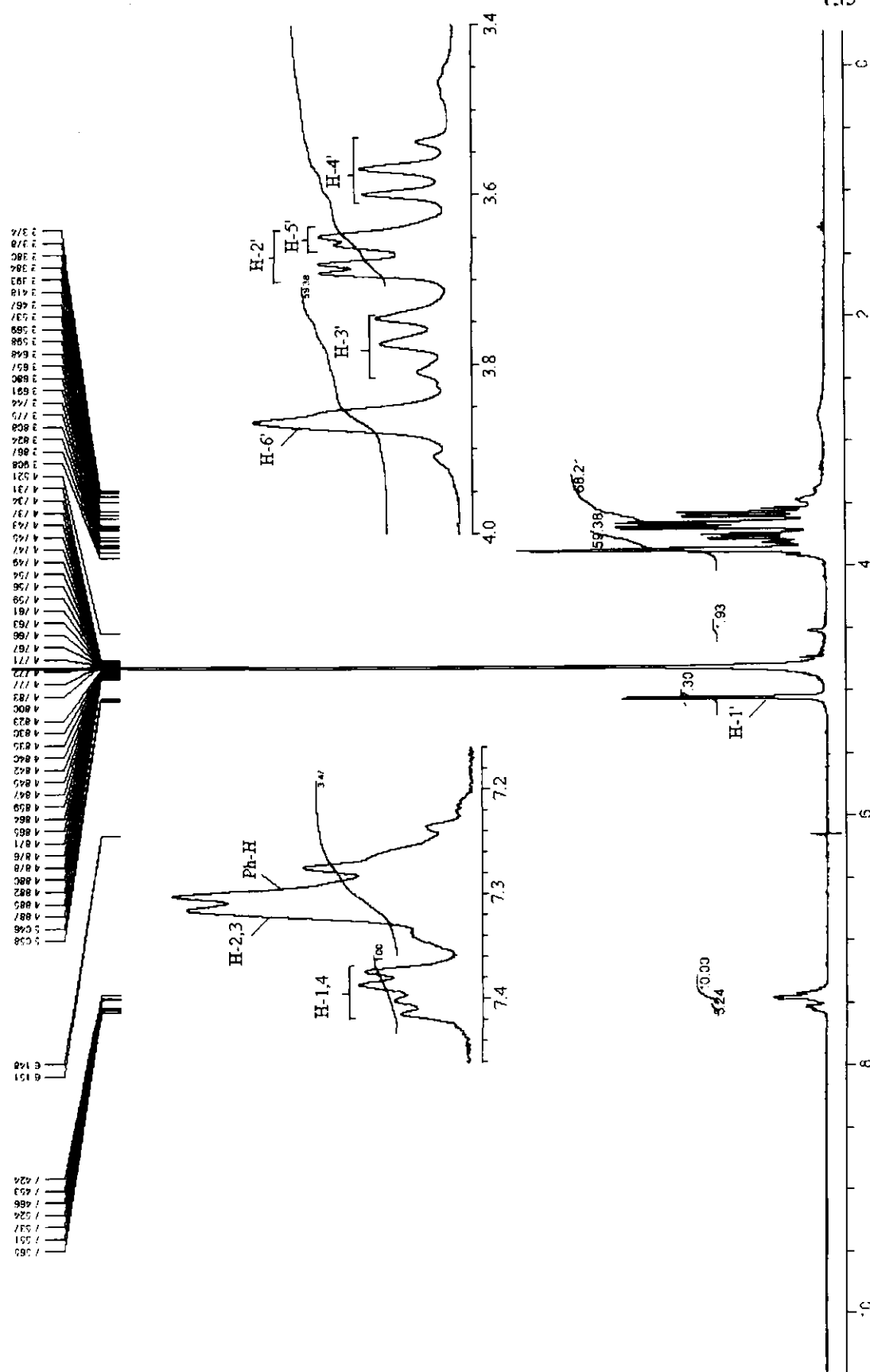


Fig. 55 ^1H NMR spectrum of a mixture of hydroxyzine hydrochloride and β -CD having a molar ratio $([\beta\text{-CD}]/[\text{HYZ}])$ equal to 1.56.

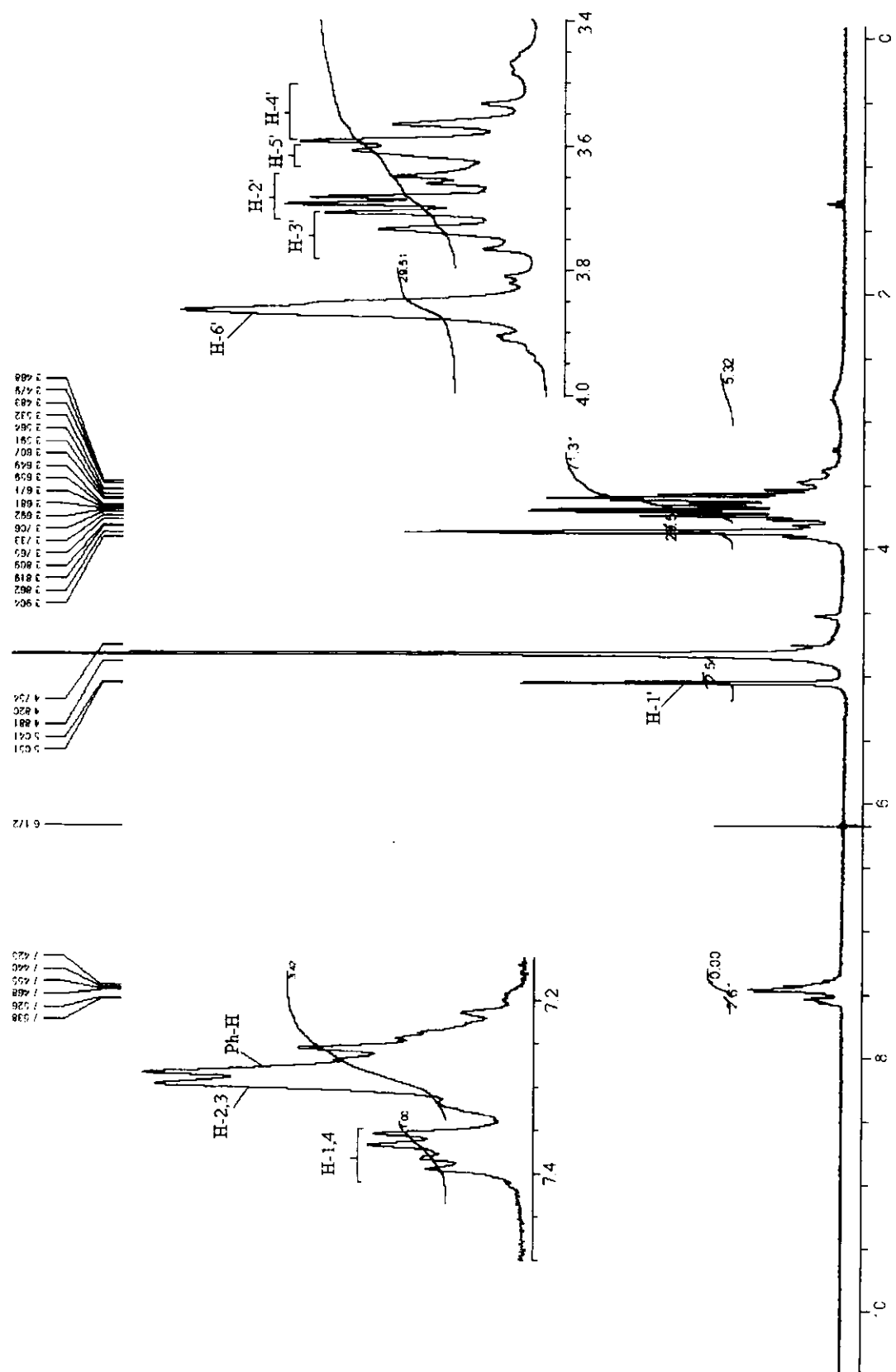


Fig. 56 ^1H NMR spectrum of a mixture of hydroxyzine hydrochloride and β -CD having a molar ratio $([\beta\text{-CD}]/[\text{HYZ}])$ equal to 1.00.

Result and Discussion

¹H NMR Chemical Shift Change Data of β -CD

The assignment of β -CD cavity protons was made with the help of COSY spectral data. The signal for H-1' appears separate from rest of the β -CD protons, helping in the identification of all the β -CD protons. The β -CD cavity protons, namely H-3' and H-5', displayed significant highfield shift changes in the presence of HYZ which increased with the increase in the concentration of HYZ. Small chemical shift changes were observed in the H-6' while other β -CD protons exhibited negligible shift changes. Fig. 57 shows changes in the chemical shifts of β -CD protons in the presence of HYZ compared to pure β -CD. The chemical shift changes for all the β -CD protons in the presence of HYZ are given in Table 13. The highfield shift changes in the β -CD cavity protons can only be attributed to the inclusion of the HYZ into the β -CD cavity resulting in the formation of HYZ- β -CD complex/es, in analogy to earlier studies.^{233, 237, 261, 262}

Table 13 ¹H NMR chemical shift changes ($\Delta\delta$) for the β -CD protons upon complexation.

Sample	$[\beta\text{-CD}]$ /[HYZ]	H-1'	H-2'	H-3'	H-4'	H-5'	H-6'
A	2.88	0.0164	0.0408	-0.0945	0.0211	-0.0905	-0.0121
B	2.50	0.0164	0.0433	-0.1055	0.0161	-0.1012	-0.0136
C	2.18	0.0134	0.0403	-0.1065	0.0131	-0.1130	-0.0196
D	1.56	0.0064	0.0438	-0.1645	0.0071	-0.1467	-0.0226
E	1.00	0.0032	0.0473	-0.2135	0.0021	-0.1977	-0.0266

Negative values indicate highfield shift.

(Temperature = 25 °C, Solvent = D₂O)

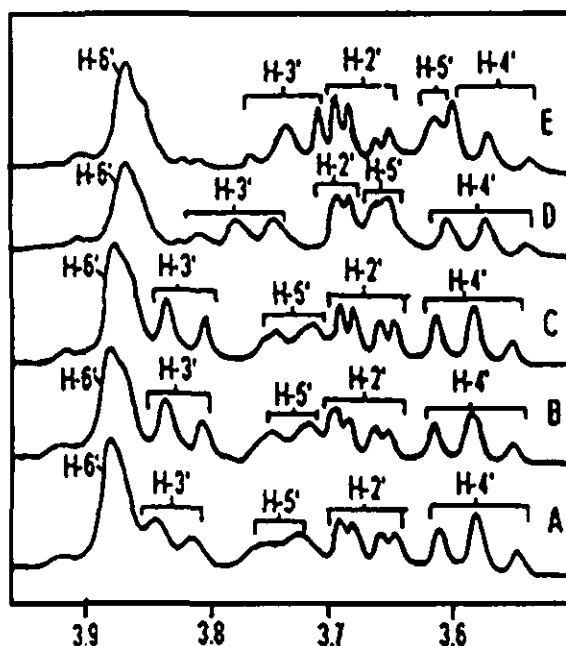


Fig. 57 Part of ¹H NMR spectra (300 MHz) showing signals for β-CD protons in the presence of varying amounts of HYZ ([β-CD]/[HYZ] ratios (A) 2.88 (B) 2.50 (C) 2.18 (D) 1.56 (E) 1.00. (Temperature = 25 °C, Solvent = D₂O)

Stoichiometry of the HYZ-β-CD Complex

The stoichiometry and the association constant (K_a) of the complex/es were determined by Scott method.²⁵⁶

$$[G]/\Delta\delta_{\text{obs}} = [G]/\Delta\delta_{\text{max}} + \Delta\delta_{\text{max}}/K_a$$

The plot of $\Delta\delta_{\text{H-5'}}$ against [HYZ] in the form of $[\text{HYZ}]/\Delta\delta_{\text{obs}}$ verses [HYZ] gave excellent linear fit (Fig. 58) confirming 1:1 stoichiometry for the HYZ-β-CD complex. The slope

of the plot is thus equal to $1/\Delta\delta_{\max}$ and the intercept with the vertical axis equal to $\Delta\delta_{\max}/K_a$, allowing the estimation of K_a to be 58 M^{-1} .

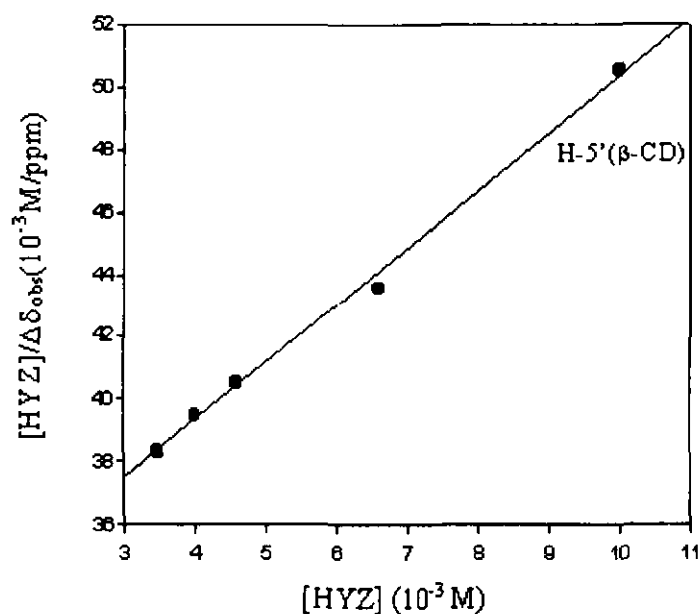


Fig. 58 Scott plot of $[\text{HYZ}]/\Delta\delta_{\text{H-5'}}$ versus $[\text{HYZ}]$ for 1:1 HYZ- β -CD complex.

¹H NMR Assignment of Hydroxyzine Hydrochloride

The signal for benzylic proton was observed at 4.981 in the spectrum of pure HYZ while signals for all the remaining aliphatic protons appeared in the region 3.00-4.00. The signals for the five phenyl ring protons and four p-chlorophenyl ring protons appeared separately in the absence of β -CD. The phenyl ring protons appeared as a multiplet in the region 7.20-7.35 while p-chlorophenyl protons were observed as two partly merged doublets in the region 7.35-7.45 (Fig. 51).

¹H NMR Chemical Shift Change Data of Hydroxyzine Hydrochloride

In the presence of β -CD, the benzylic proton showed highfield shift and appeared partly merged with the HDO signal. The remaining aliphatic protons appeared merged with the β -CD protons and could not be studied. The signal for two protons of p-chlorophenyl ring moved highfield, in the presence of β -CD, and appeared merged with phenyl ring protons as indicated by the integration of the signals (Figs. 52-56). Moreover, there were significant changes in the shape of signal for phenyl ring protons, but the resolution of the peaks was not good on 300 MHz instrument. To clearly establish which of the two p-chlorophenyl ring protons moved highfield, a NOESY spectrum was recorded for a mixture of HYZ and β -CD ($[\beta\text{-CD}]/[\text{HYZ}]=0.58$) on a 600 MHz instrument (Fig. 59). The spectrum showed phenyl ring protons as a triplet at 7.322 (1H) and a multiplet at 7.374 (4H) while the p-chlorophenyl ring protons were observed as two multiplets, each integrating for two protons, at 7.449 and 7.469. The signal at 7.469 exhibited cross connection peak with the benzylic proton, which appeared partly merged with HDO peak, in the NOESY spectrum and therefore was assignable to H-1, 4 while the signal which moved highfield in the presence of β -CD could only be assigned to H-2, 3. The changes in the shapes and chemical shifts of both the aromatic rings protons indicate the involvement of both these rings in the complexation with β -CD. Fig. 60 shows the aromatic region of the spectra of HYZ, in the absence as well as in the presence of β -CD.

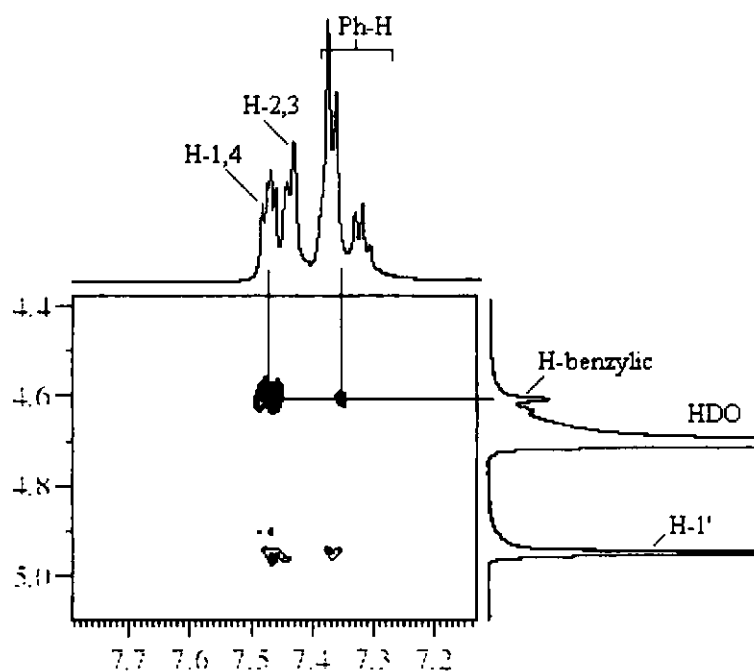


Fig. 59 A part of NOESY (600 MHz) spectrum of a mixture of HYZ and β -CD showing dipolar interactions between benzylic and H-1, 4 protons of HYZ.

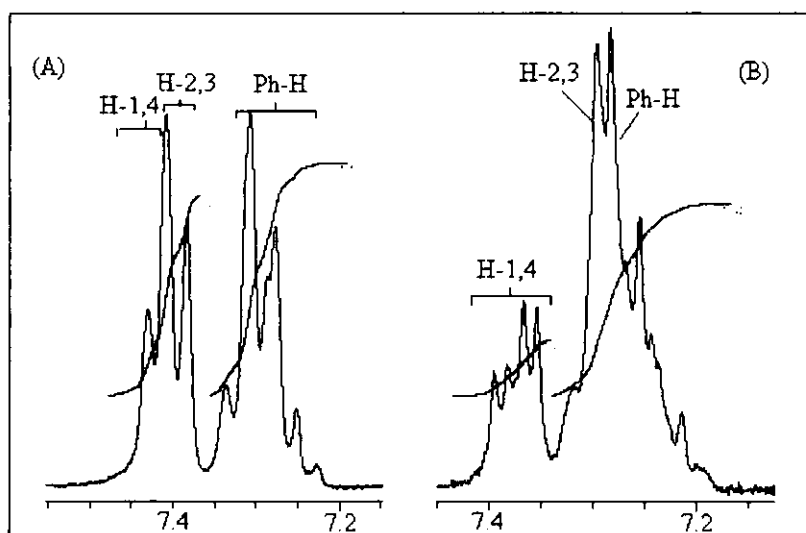


Fig. 60 Part of the ^1H NMR (300 MHz) spectra showing aromatic protons of HYZ (A) pure HYZ (B) mixture of HYZ and β -CD ($[\beta\text{-CD}]/[\text{HYZ}]=2.88$).

ROESY Data of Hydroxyzine Hydrochloride- β -CD Mixture

The ROESY²³² spectrum of a mixture of HYZ and β -CD ($[\beta\text{-CD}]/[\text{HYZ}]=0.58$) on a 600 MHz instrument (Fig. 61) exhibited strong cross correlation peaks between H-3' and H-5', positioned inside the β -CD cavity, and protons of both the aromatic rings of HYZ confirming the penetration of both the aromatic rings into the β -CD cavity. Moreover, strong cross peaks between H-6' of β -CD and phenyl ring protons were also observed. The equally strong interactions of H-1, 4 and H-2, 3 of p-chlorophenyl ring with H-3' and H-5' of β -CD are only possible if p-chlorophenyl ring penetrates the cavity from both the sides giving rise to two 1:1 inclusion complexes. Similarly, the interaction of all the phenyl ring protons with H-3', 5' and 6' at the same time can only be explained if phenyl ring approaches the β -CD cavity from both the sides.

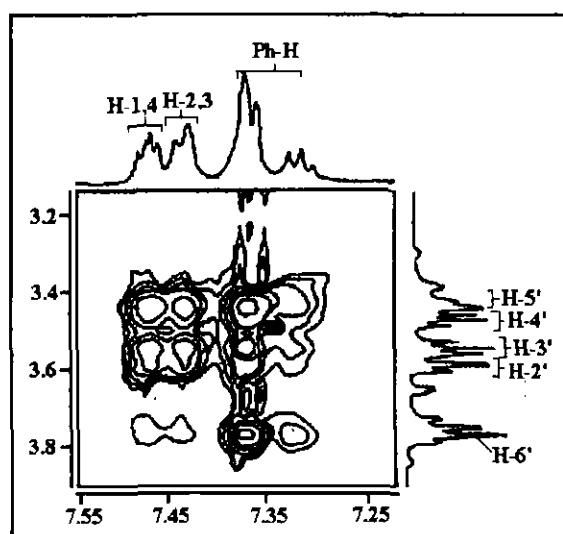


Fig. 61 Part of the 2D ROESY (600 MHz) spectrum of a mixture of β -CD and HYZ showing cross peaks between aromatic protons of HYZ and cavity protons of β -CD. (Mixing time = 0.225 sec, Delay Time = 2.8 sec)

Thus, taking into consideration the ROESY spectral data and 1:1 stoichiometry, in conjunction with the chemical shift changes observed in the ^1H NMR spectra, it can be said that HYZ forms four 1:1 inclusion complexes with β -CD, in aqueous solution, by the penetration of the aromatic rings from both the sides of the β -CD cavity. The highfield shift changes in the H-2, 3 can be explained in terms of hydrogen bonding between the Cl atom and H atom of the rim hydroxyl groups. The structures for all the 1:1 HYZ- β -CD complexes are proposed as shown in Fig. 62.

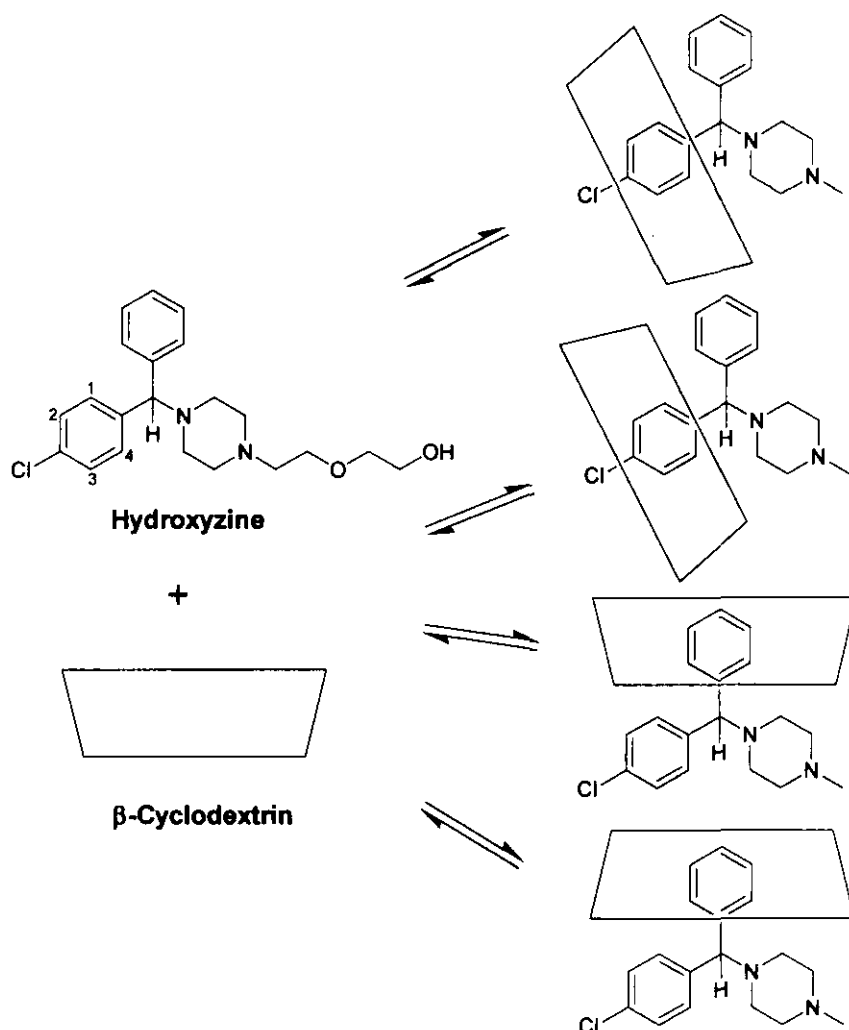
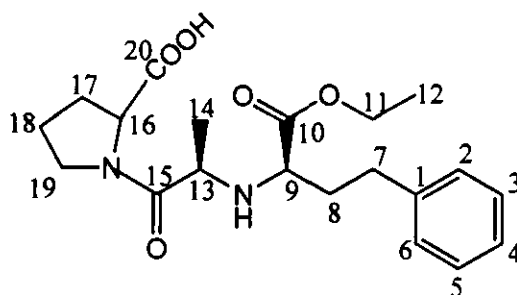


Fig. 62 Proposed model of the inclusion equilibria for all the 1:1 HYZ- β -CD complexes.

*¹H NMR Spectroscopic Study of
Complexation of Enalapril Maleate
with β -Cyclodextrin*

Enalapril maleate (EN), chemically known as 1-{N-[(S)-1-ethoxycarbonyl-3-phenylpropyl]-L-alanyl}-L-proline maleate, and its analogs are highly effective antihypertensive agents which act as angiotensin converting enzyme (ACE) inhibitors. ACE inhibitors prevent conversion of angiotensin I to II and act to widen the blood vessels resulting in easier pumping of blood by the heart. They are, therefore, used for the treatment of hypertension, congestive heart failure and to alleviate strain on heart damaged by heart attack.^{267, 268}



Enalapril

Experimental

All the ^1H NMR spectra (Figs. 63-68) were recorded on a JEOL 400 MHz instrument in D_2O at 25 $^\circ\text{C}$. The chemical shift values are reported in δ (ppm) with reference to HDO peak at 4.7846. ^1H NMR chemical shift change data was obtained for β -CD protons, to determine the stoichiometry of the inclusion complex, by keeping the concentration of the β -CD constant at 10 mM while the concentration of EN was varied from 11.4 to 52.6 mM. The molar ratios ($[\beta\text{-CD}]/[\text{EN}]$) were calculated by direct integration of appropriate signals. The COSY (Fig. 69) and ROESY (Fig. 70) spectra were recorded for a mixture of EN and β -CD. As expected, all the spectra consisted of one set of concentration dependent resonances for each proton or group of equivalent protons indicating a fast reversible exchange between free and complexed EN on the NMR time scale.

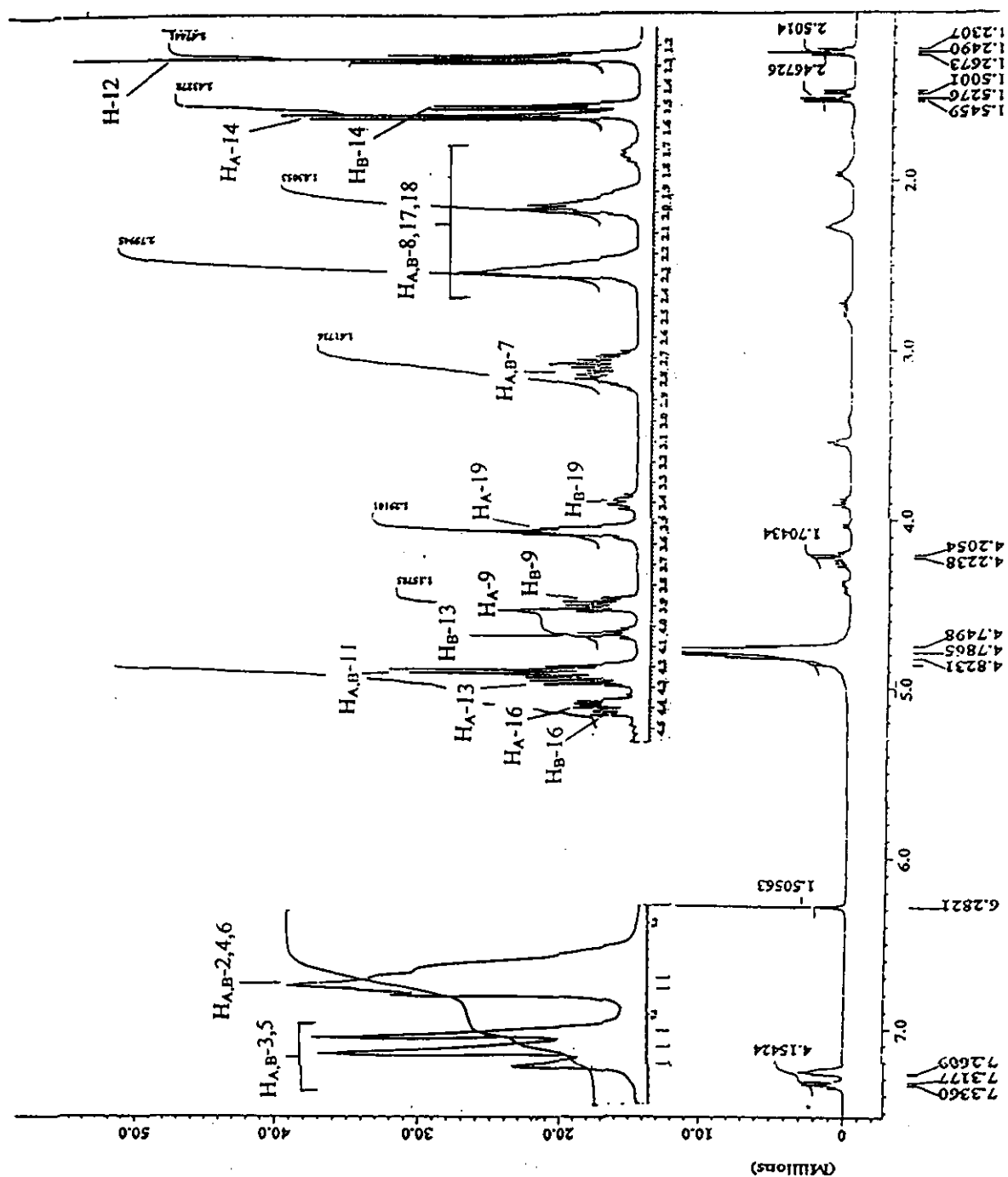


Fig. 63 ^1H NMR spectrum (400 MHz) of pure enalapril maleate.

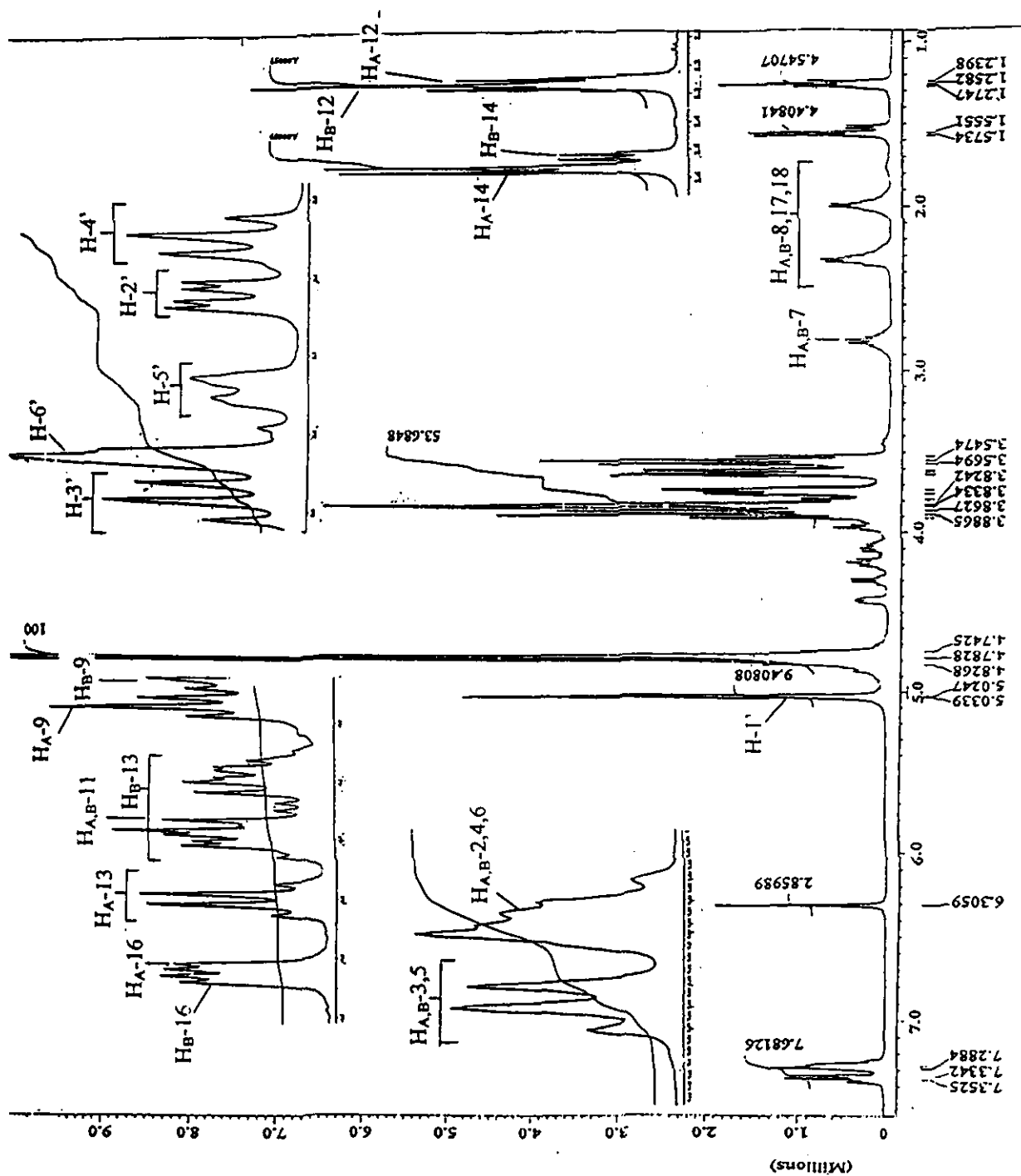


Fig. 64 ^1H NMR spectrum (400 MHz) of a mixture of enalapril maleate and β -CD having a molar ratio ($[\beta\text{-CD}]/[\text{EN}]$) equal to 0.875.

Fig. 65 ^1H NMR spectrum (400 MHz) of a mixture of enalapril maleate and β -CD having a molar ratio ($[\beta\text{-CD}]/[\text{EN}]$) equal to 0.750.

Fig. 66 ^1H NMR spectrum (400 MHz) of a mixture of enalapril maleate and β -CD having a molar ratio ($[\beta\text{-CD}]/[\text{EN}]$) equal to 0.560.

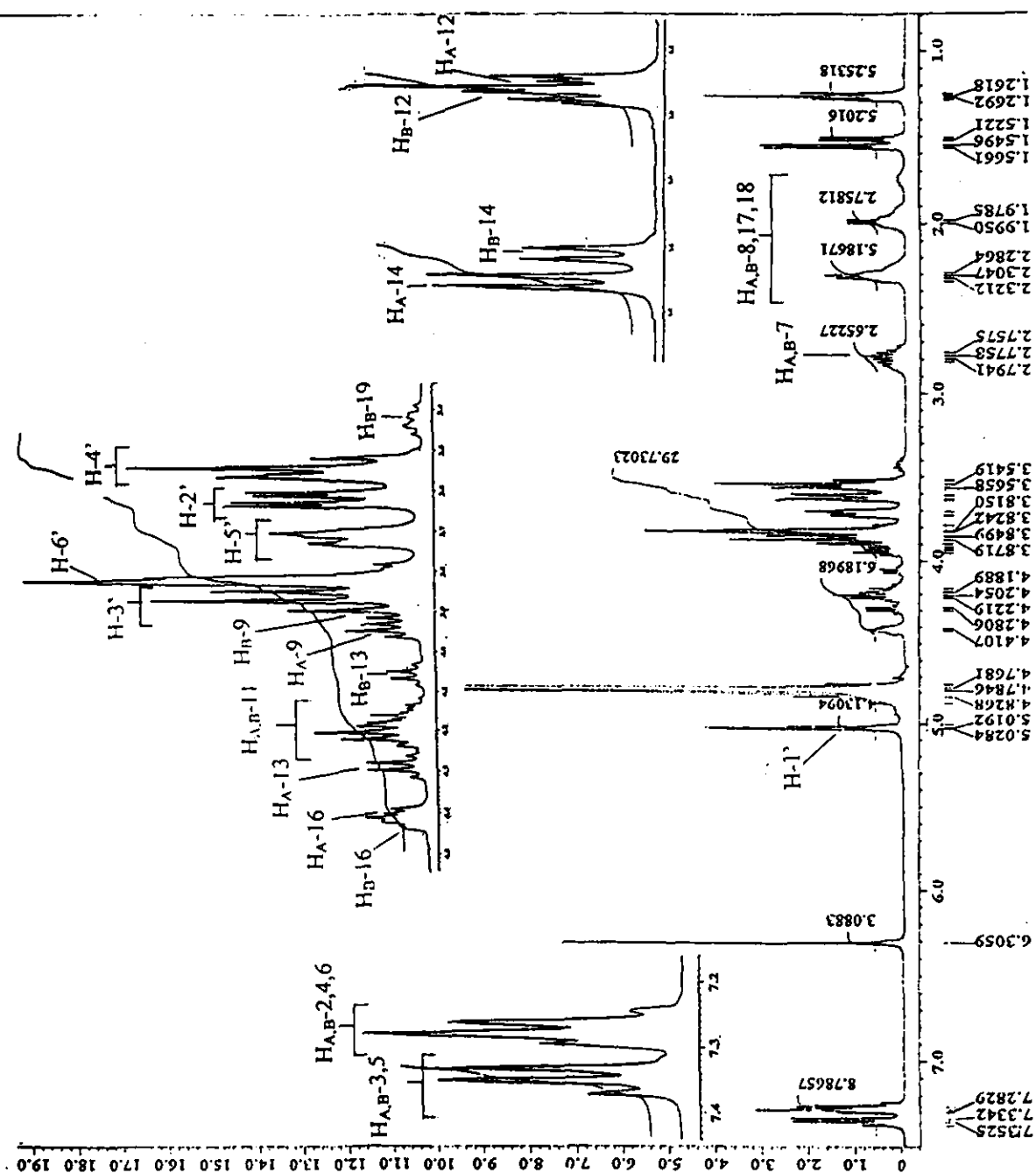


Fig. 67 ^1H NMR spectrum (400 MHz) of a mixture of enalapril maleate and β -CD having a molar ratio ($[\beta\text{-CD}]/[\text{EN}]$) equal to 0.335.

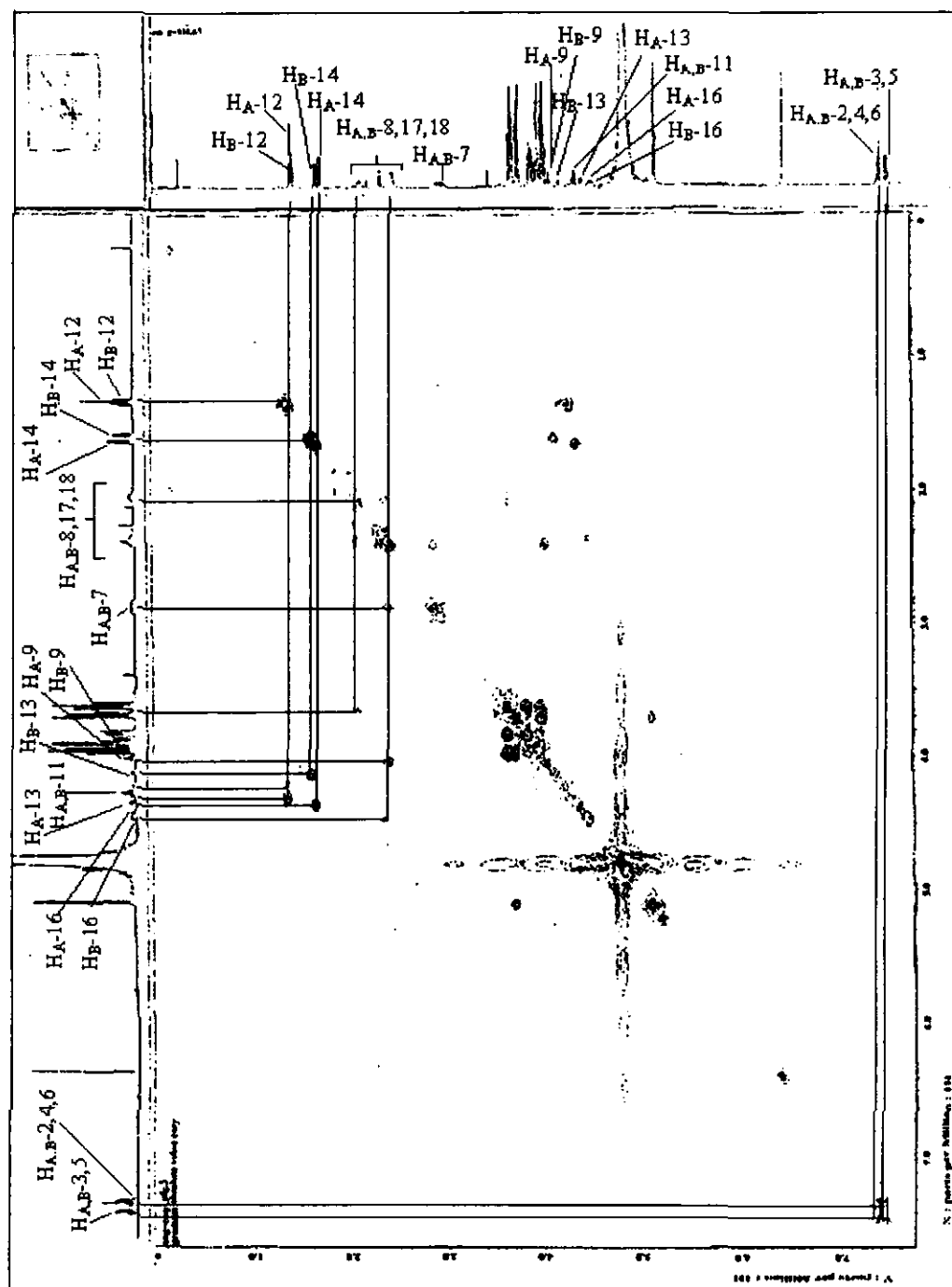


Fig. 69 2D COSY spectrum of a mixture of EN and β -CD.

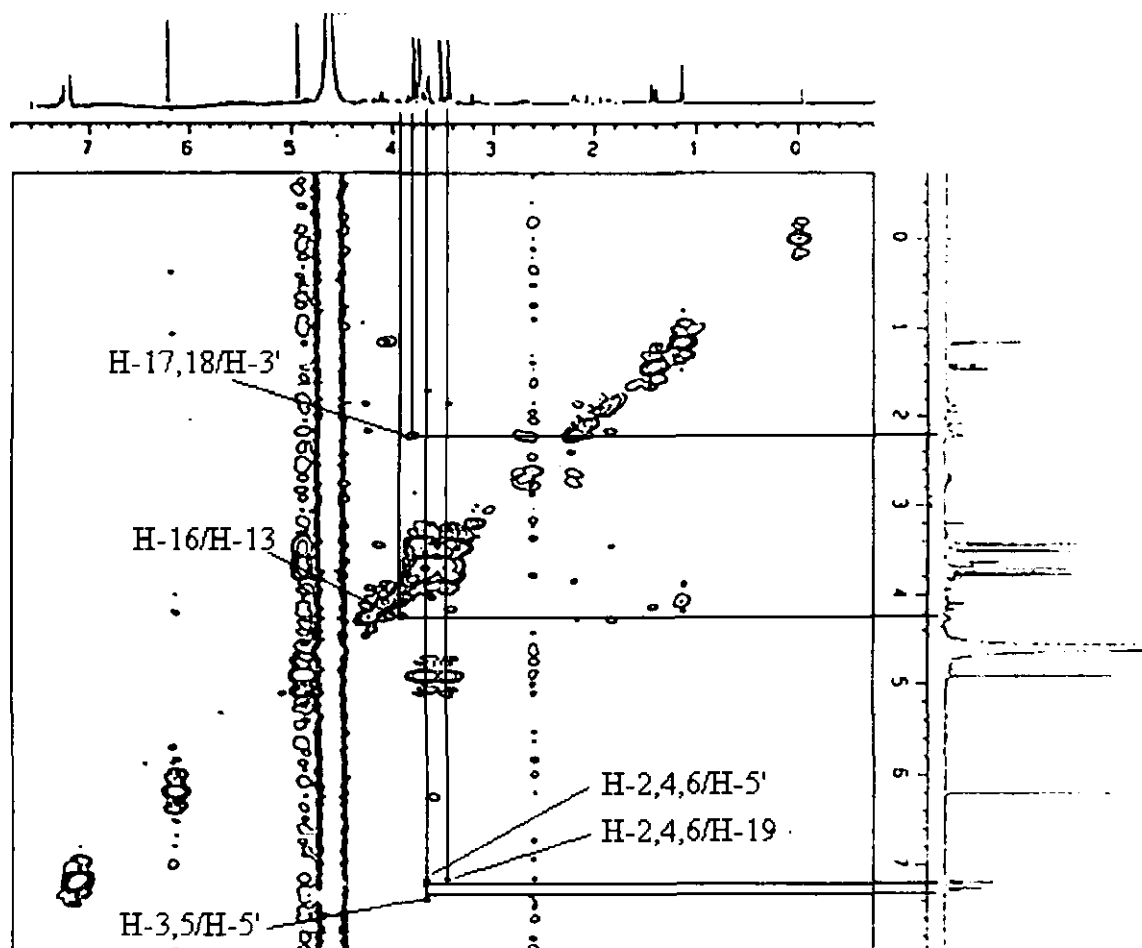


Fig. 70 2D ROESY spectrum of a mixture of EN and β -CD.

Result and Discussion

^1H NMR Chemical Shift Change Data of β -CD

The β -CD cavity protons were easily identified by their characteristic shapes and chemical shift values and the assignment is fully supported by COSY spectral data

(Fig. 71). Significant highfield shift changes were observed in the protons situated inside the β -CD cavity, namely H-3' and H-5', in the presence of EN which increased with the increasing concentration of EN. Other β -CD protons did not show any significant chemical shift changes in the presence of EN. Fig. 72 shows changes in the chemical shifts of β -CD protons in the presence of EN compared to pure β -CD while the ^1H NMR chemical shift change data for all the β -CD protons is given in Table 14. The highfield shift changes in the β -CD cavity protons confirmed the inclusion of the EN into the β -CD cavity resulting in the formation of β -CD-EN complex, as reported earlier.^{233, 237, 261-262}

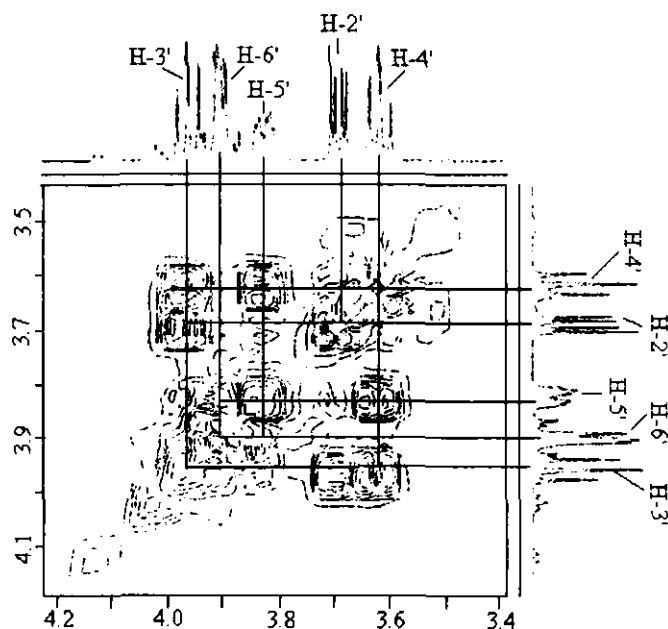


Fig. 71 A part of COSY spectrum (400 MHz) of EN and β -CD mixture showing region containing β -CD signals.

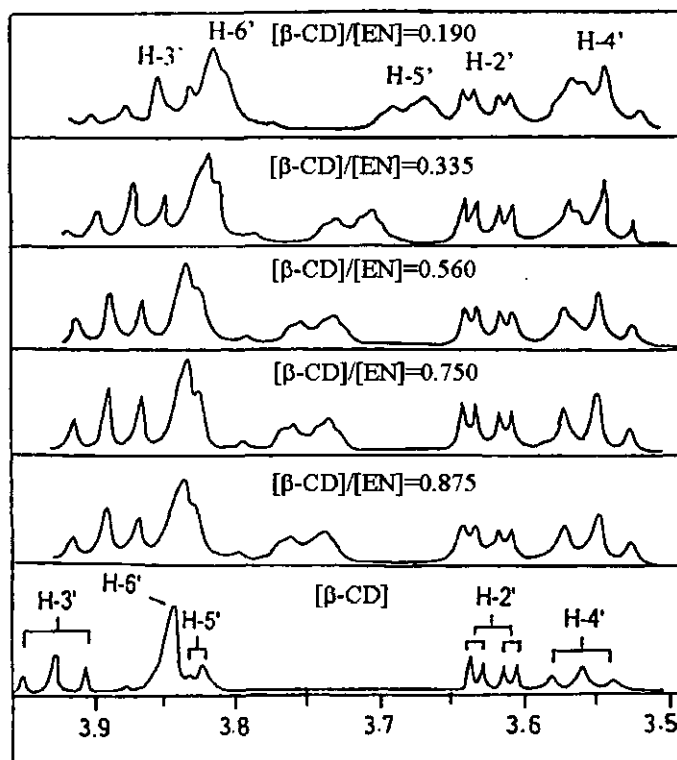


Fig. 72 Partial ^1H NMR (400 MHz) spectra showing signals for the protons of β -CD in the absence as well as in the presence of varying amounts of EN. (Temperature = 25 $^\circ\text{C}$, Solvent = D_2O)

Table 14 ^1H NMR (400 MHz) chemical shift change ($\Delta\delta$) values for β -CD protons in the presence of EN in D_2O . (Temperature = 25 $^\circ\text{C}$)

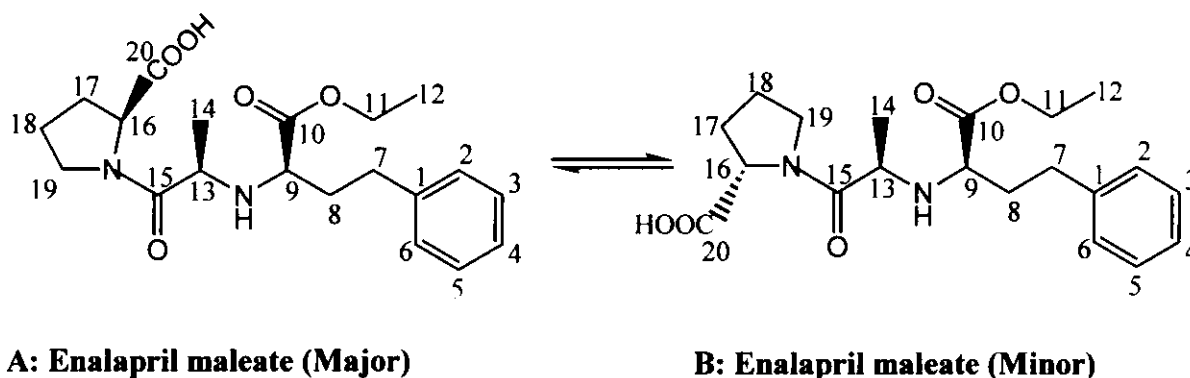
A/B [#]	[β -CD]/ [EN]	H-1'	H-2'	H-3'	H-4'	H-5'	H-6'
73:27	0.875	-0.0055	0.0100	-0.0402	-0.0019	-0.0730	-0.0073
60:40	0.750	-0.0073	0.0064	-0.0403	-0.0037	-0.0738	-0.0091
61:39	0.560	-0.0091	0.0046	-0.0439	-0.0055	-0.0798	-0.0128
59:41	0.335	-0.0128	0.0046	-0.0567	-0.0092	-0.0998	-0.0183
64:36	0.190	-0.0201	0.0037	-0.0751	-0.0147	-0.1428	-0.0311

[#]cis /trans ratio of EN.

Negative values indicate high field shift.

¹H NMR Assignment of Enalapril Maleate

Two diastereomeric forms, (SSS)- and (RSS)-, of enalapril maleate (EN) are known of which only (SSS)-diastereomer is used as drug.²⁶⁹ ¹H NMR spectra of EN (SSS-diastereomer), in the absence as well as in the presence of β -CD, showed that two geometrical isomers are in equilibrium in solution as some of the non-aromatic protons appeared separately for two isomers. Cis-trans isomerism is possible in the case of EN due to hindered rotation along amide bond.²⁷⁰ The spectral assignment for the major (A) and minor (B) isomers was made with the help of COSY (Fig. 69) and ROESY (Fig. 70) spectral data. The chemical shift data of the studied protons for pure EN is given in Table 15.



The signals for the protons of three methylenic groups, for two isomers, at C-8, 17, 18 were found resonating as multiplets centered at 2.2700 ($3H_A+4H_B$), 1.9600 ($3H_A+1H_B$) and 1.7200 ($1H_B$). The signal for H_A -19 was observed resonating at 3.5328 in the case of pure EN. In the presence of β -CD, H_A -19 signal merged with β -CD protons and, therefore, its chemical shift changes could not be determined. The structures for the two

isomers could be established by comparative study of the H-13, 14 and 16 signals. The major isomer was assigned structure (A) since H-13 and H-14, being in close proximity of oxygen of -COOH group should appear downfield compared to signals for the same protons of the minor isomer (B) which is in good agreement with earlier observation.²⁷¹ As expected, the signal for H-16 for major isomer (A) appears relatively upfield compared to that of minor isomer. The ^1H NMR assignment of major and minor isomers is also supported by the through space interaction between H-16 and H-13 signals, in the case of major isomer, in the ROESY spectrum.

^1H NMR Chemical Shift Change Data of Enalapril Maleate

As expected, changes in the shapes as well as chemical shifts of EN protons were observed in the presence of β -CD. All the protons, except H-11, exhibited downfield shift changes. The highfield shift in the H-11 signal may be due to hydrogen bonding between the hydrogen of the rim hydroxyl and $\text{-COOC}_2\text{H}_5$ group. The signal for H-11, which appeared as a quartet at 4.2321 in the spectrum of pure EN, became complex in the presence of β -CD making it difficult to calculate its chemical shift changes. The $\Delta\delta$ values for all the studied protons of the EN are given in Table15.

Table 15 ^1H NMR (400 MHz) data for EN and its chemical shift change ($\Delta\delta$) data in the presence of β -CD in D_2O . (Temperature = 25 $^\circ\text{C}$,

Proton	[EN]	$[\beta\text{-CD}]/$ [EN]=0.875	$[\beta\text{-CD}]/$ [EN]=0.750	$[\beta\text{-CD}]/$ [EN]=0.560	$[\beta\text{-CD}]/$ [EN]=0.335	$[\beta\text{CD}]/$ [EN]=0.190
H _A -12	1.2490	0.0092	0.0110	0.0110	0.0128	0.0092
H _B -12	1.2490	0.0183	0.0202	0.0202	0.0202	0.0147
H _B -14	1.4910	0.0302	0.0284	0.0247	0.0229	0.0174
H _A -14	1.5368	0.0275	0.0257	0.0232	0.0211	0.0156
H _{A,B} -7	2.7600	0.0700	0.0620	0.0500	0.0450	0.0350
H _B -19	3.4000	0.0300	0.0200	0.0150	0.0100	0.0020
H _B -9	3.8700	0.0530	0.0476	0.0421	0.0312	0.0310
H _A -9	3.9100	0.0608	0.0517	0.0462	0.0388	0.0278
H _B -13	4.0380	#	0.0290	0.0250	0.0220	0.0170
H _A -13	4.2620	0.0360	0.0333	0.0296	0.0280	0.0220
H _A -16	4.3800	0.0436	0.0300	0.0280	0.0262	0.0230
H _B -16	4.4200	0.0130	0.0100	0.0080	0.0050	0.0015
H _{A,B} -2,4,6	7.2550	0.0250	0.0200	0.0150	0.0120	0.0080
H _{A,B} -3,5	7.3360	0.0140	0.0140	0.0100	0.0090	0.0040

#could not be determined.

Stoichiometry of the EN- β -CD Complex

Scott plot of $\Delta\delta_{\text{H-5'}}$ against [EN] in the form of $[\text{EN}]/\Delta\delta_{\text{H-5'}}$ verses [EN] was found to be a nonlinear relationship which indicated that the stoichiometry of the complex is not 1:1. The stoichiometry of the EN- β -CD complex was then determined by the mole ratio method.²⁵⁰ The ^1H NMR shift change data was obtained for β -CD protons in the presence of EN by keeping the concentration of β -CD constant while varying the concentration of

EN. The plot of $\Delta\delta_{\text{H-5'}}$ versus $[\beta\text{-CD}]/[\text{EN}]$ (Fig. 73) shows maximum around 0.5 suggesting a 1:2 stoichiometry for the $\beta\text{-CD-EN}$ complex. The stability constant of the complex was determined by the modified Scott equation for 1:2 complex.

$$[\text{G}]^2/\Delta\delta_{\text{obs}} = [\text{G}]^2/\Delta\delta_{\text{max}} + \Delta\delta_{\text{max}}/K_a$$

A plot of $\Delta\delta_{\text{H-5'}}$ against $[\text{EN}]$ in the form of $[\text{EN}]^2/\Delta\delta_{\text{H-5'}}$ versus $[\text{EN}]^2$ gave a linear fit (Fig. 74) confirming 1:2 stoichiometry for $\beta\text{-CD-EN}$ complex. The slope of the plot is thus equal to $1/\Delta\delta_{\text{max}}$ and the intercept with the vertical axis equal to $\Delta\delta_{\text{max}}/K_a$ allowing the estimation of K_a to be 340 M^{-1}

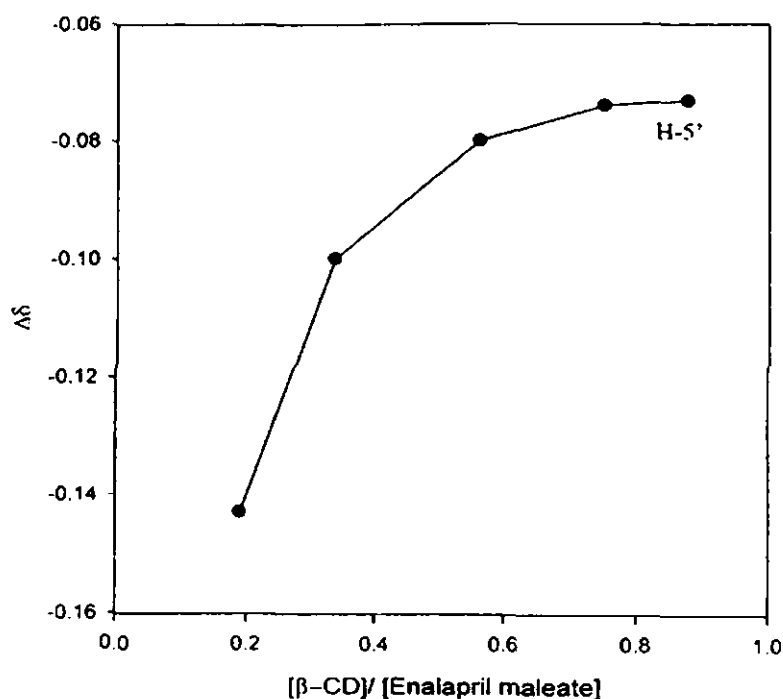


Fig. 73 Mole ratio plot of the chemical shift changes of H-5' of $\beta\text{-CD}$ during titration with EN in D_2O .

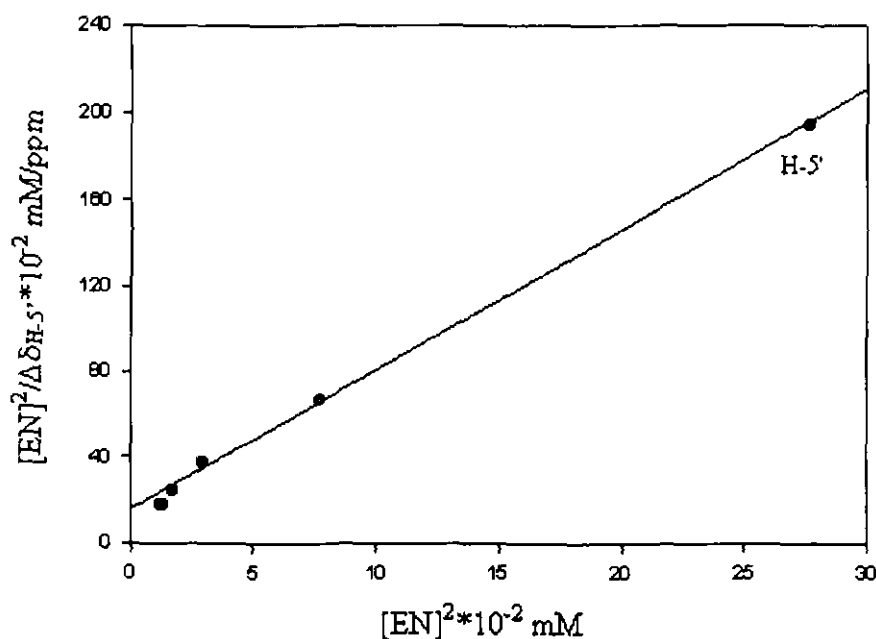


Fig. 74 Scott's plot of $[EN]^2/\Delta\delta_{H-5'}$ versus $[EN]^2$ showing 1:2 stoichiometry for β -CD-EN complex.

ROESY Spectral Data of β -CD-EN Mixture

ROESY²³² spectrum (Fig. 70) was recorded for a mixture of EN and β -CD to study the mode of penetration of two guest molecules into a β -CD cavity. Intermolecular cross peaks observed between the aromatic protons of the guest and H-5' of β -CD and no cross peaks observed between the aromatic protons and H-3' confirm the shallow penetration of the aromatic ring into the β -CD cavity from narrower rim side.²⁴³ Moreover, Cross peak between H-19 and H-2, 4, 6 of EN confirmed that aromatic ring of one guest molecule and 5-membered ring of another molecule share the same β -CD cavity which is in agreement with the 1:2 β -CD-EN stoichiometry of the complex. Cross peak between

H-17 (18) and H-3' was also observed which confirms that 5-membered ring enters the β -CD cavity from wider rim side. Fig. 75 shows an expanded region of the ROESY spectrum containing cross peaks between the β -CD and EN protons. Since EN exists as a mixture of two geometrical isomers in solution, there are several 1:2 β -CD-EN combinations possible. The general structure for the 1:2 β -CD-EN complex can be shown as in Fig. 76.

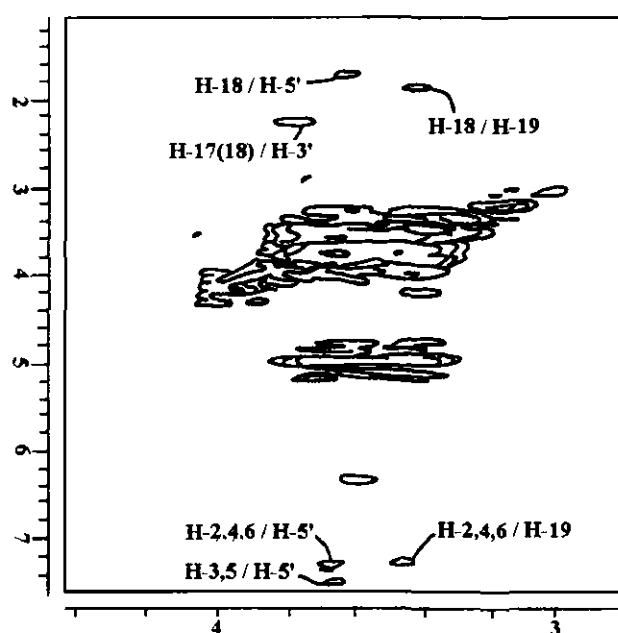


Fig. 75 Expansion of part of 2D ROESY spectrum showing cross peaks between EN and β -CD protons. (Mixing time = 0.25 sec, Delay Time = 2.35 sec)

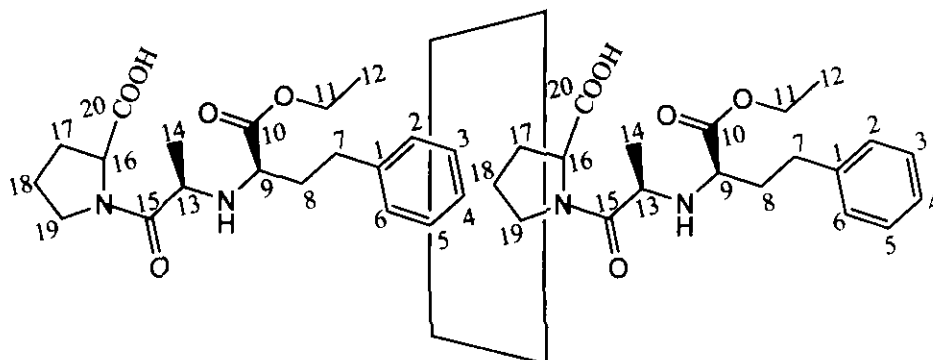
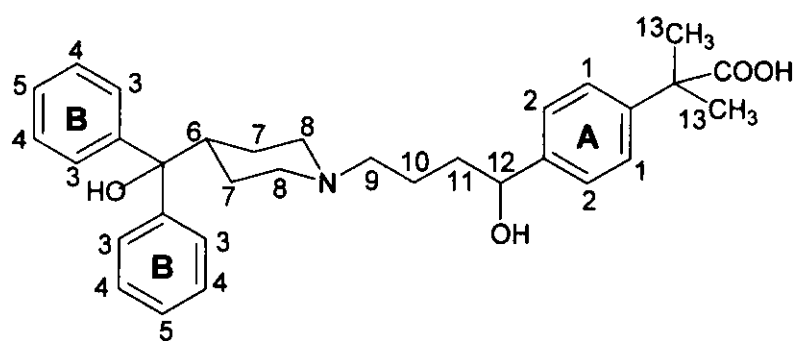


Fig. 76 Proposed structure for 1:2 EN- β -CD complex.

It is important to mention here that the cis-trans ratio for EN, as determined from the integration of 14-CH₃ doublet, remained almost unchanged, in the presence of β -CD, in four samples. However, there was a significant change observed in one of the sample (Table 14). This can only be explained in terms of catalysis by the β -CD. However, this needs further investigation.

*¹H NMR Spectroscopic Study of
Complexation of Fexofenadine
Hydrochloride with β -Cyclodextrin*

Fexofenadine hydrochloride (FFN), chemically known as (\pm) -4-{1-hydroxy-4-[4-(hydroxydiphenylmethyl)-1-piperidiny]-butyl}- α,α -dimethylbenzene acetic acid hydrochloride, is a second generation antihistamine that is used to treat allergies.²⁷² It is a racemate and exists as zwitter ion in aqueous media at physiological pH . It belongs to the group of amine compounds bearing diphenylmethyl functionality.



Fexofenadine

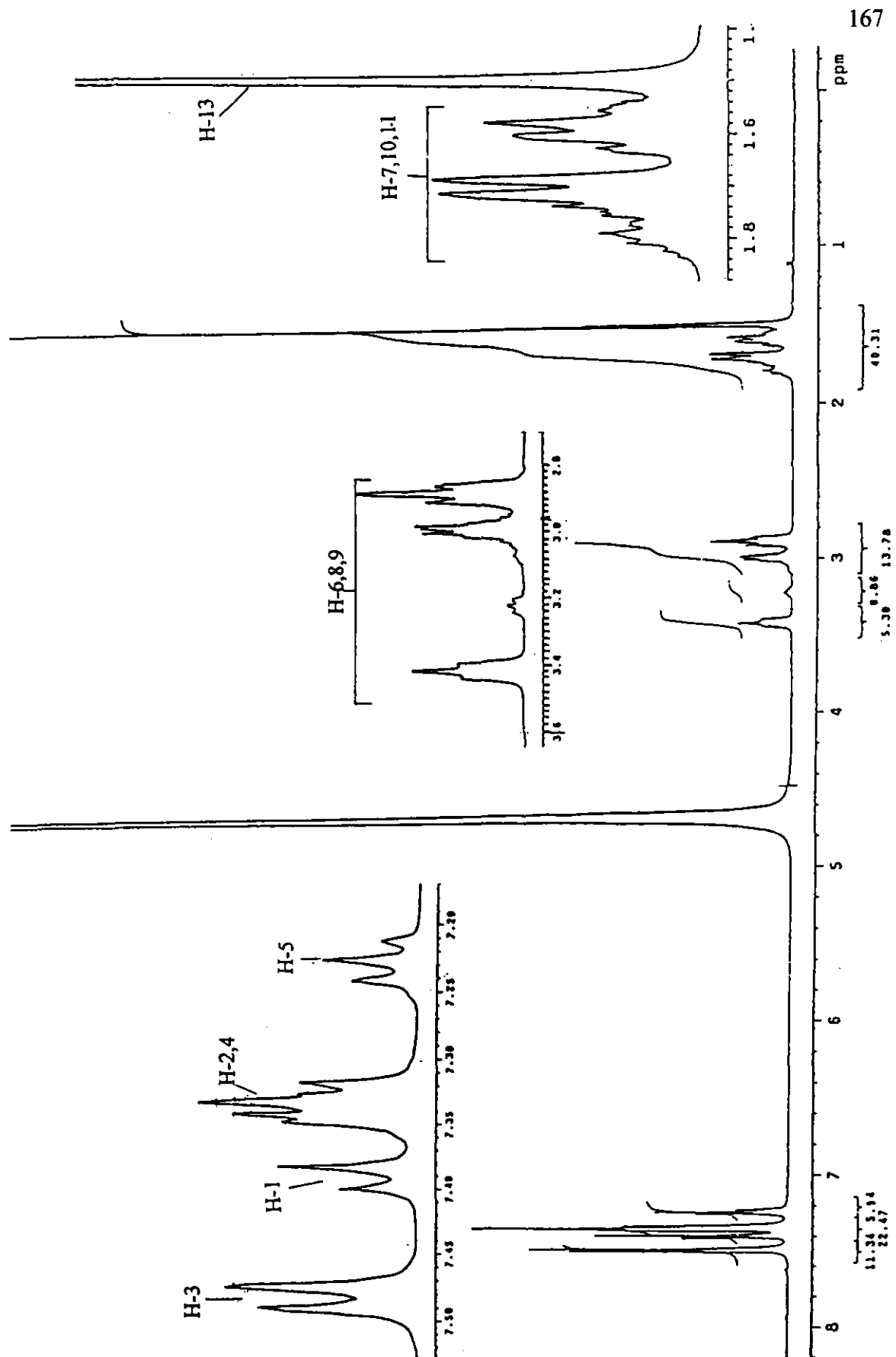


Fig. 76 ^1H NMR spectrum (500 MHz) of pure fexofenadine.

Experimental

All the ^1H NMR spectra (Figs. 77-83) were recorded on an Inova 500 MHz instrument in D_2O at 25 $^\circ\text{C}$. The chemical shift values were calculated with reference to HDO peak at 4.780 and are reported in δ (ppm). To determine the stoichiometry of the complex ^1H NMR shift data was obtained for the FFN by keeping the concentration of the FFN constant at 3.7 mM while the concentration of the β -CD was varied from 2.5-9.1 mM. The molar ratios ($[\beta\text{-CD}]/[\text{FFN}]$) were determined by direct integration of the appropriate signals. ROESY spectrum (Fig. 84) was recorded for a mixture of FFN and β -CD. All the spectra consisted of one set of concentration dependent resonances for each proton or group of equivalent protons indicating a fast reversible exchange between free and complexed FFN on the NMR time scale.

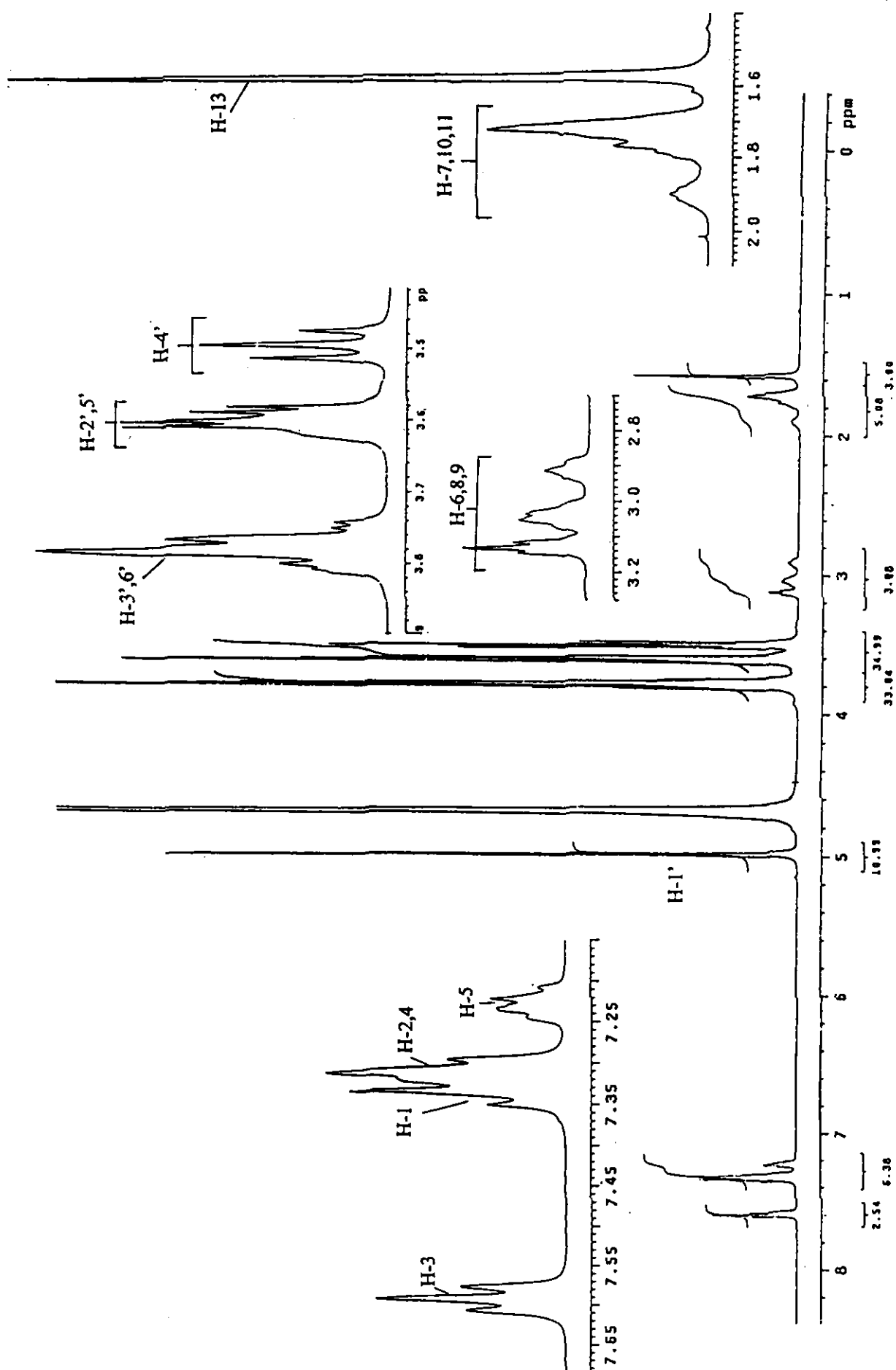


Fig. 77 ^1H NMR spectrum (500 MHz) of a mixture of fexofenadine and β -CD having a molar ratio ($[\beta\text{-CD}]/[\text{FFN}]$) equal to 2.46.

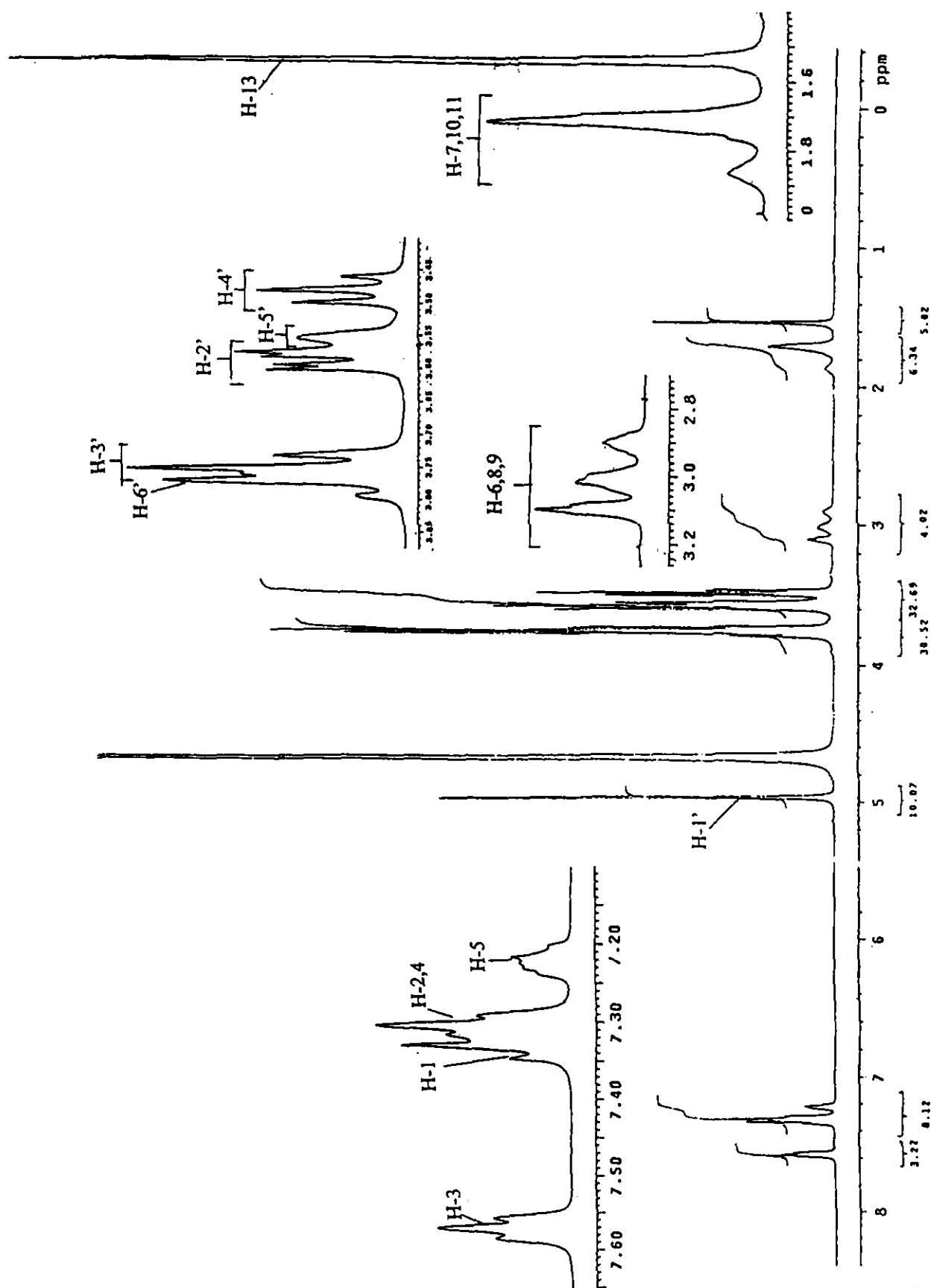


Fig. 78 ^1H NMR spectrum (500 MHz) of a mixture of fexofenadine and β -CD having a molar ratio ($[\beta\text{-CD}]/[\text{FFN}]$) equal to 1.76.

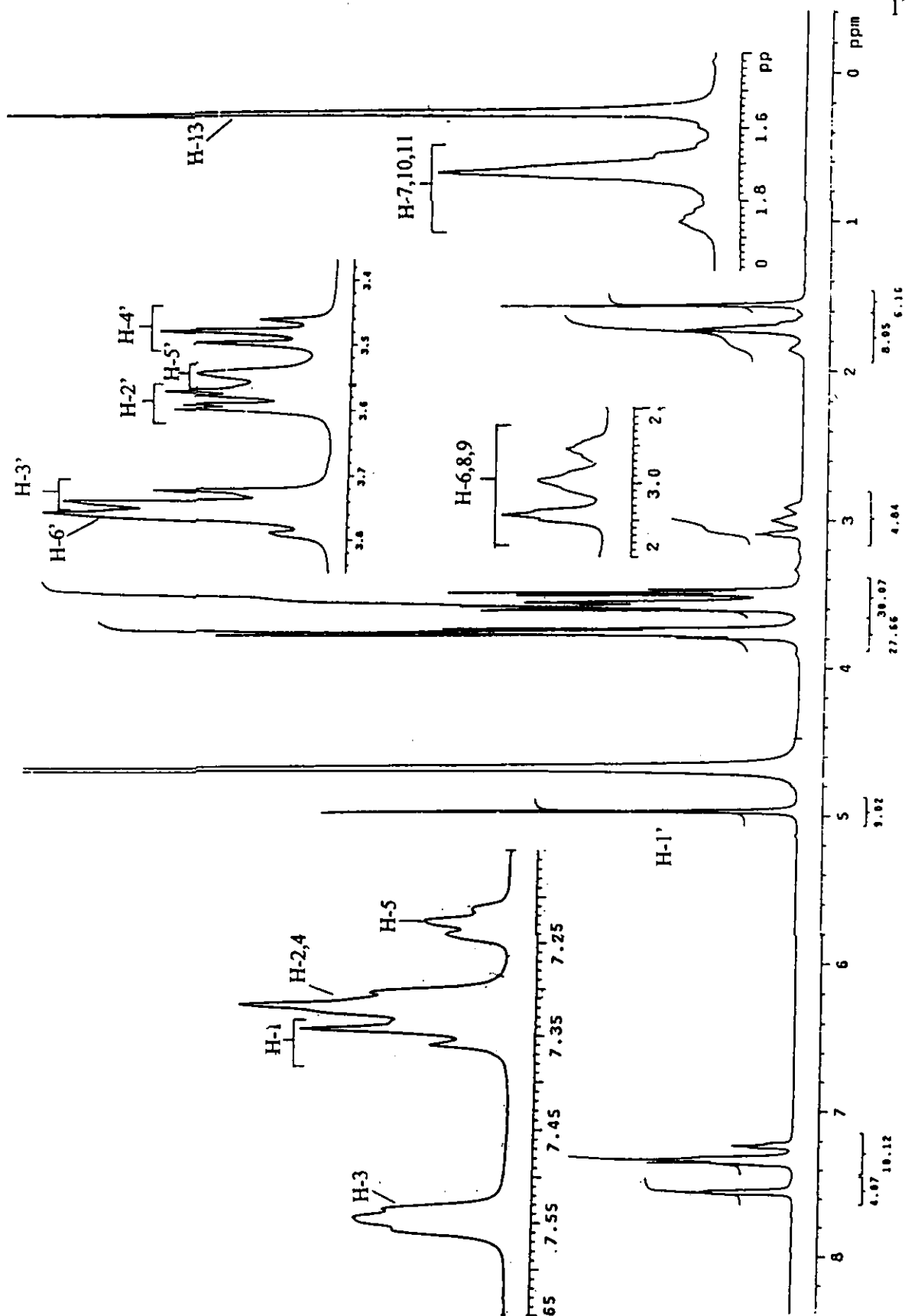


Fig. 79 ^1H NMR spectrum (500 MHz) of a mixture of fexofenadine and β -CD having a molar ratio ($[\beta\text{-CD}]/[\text{FFN}]$) equal to 1.27.

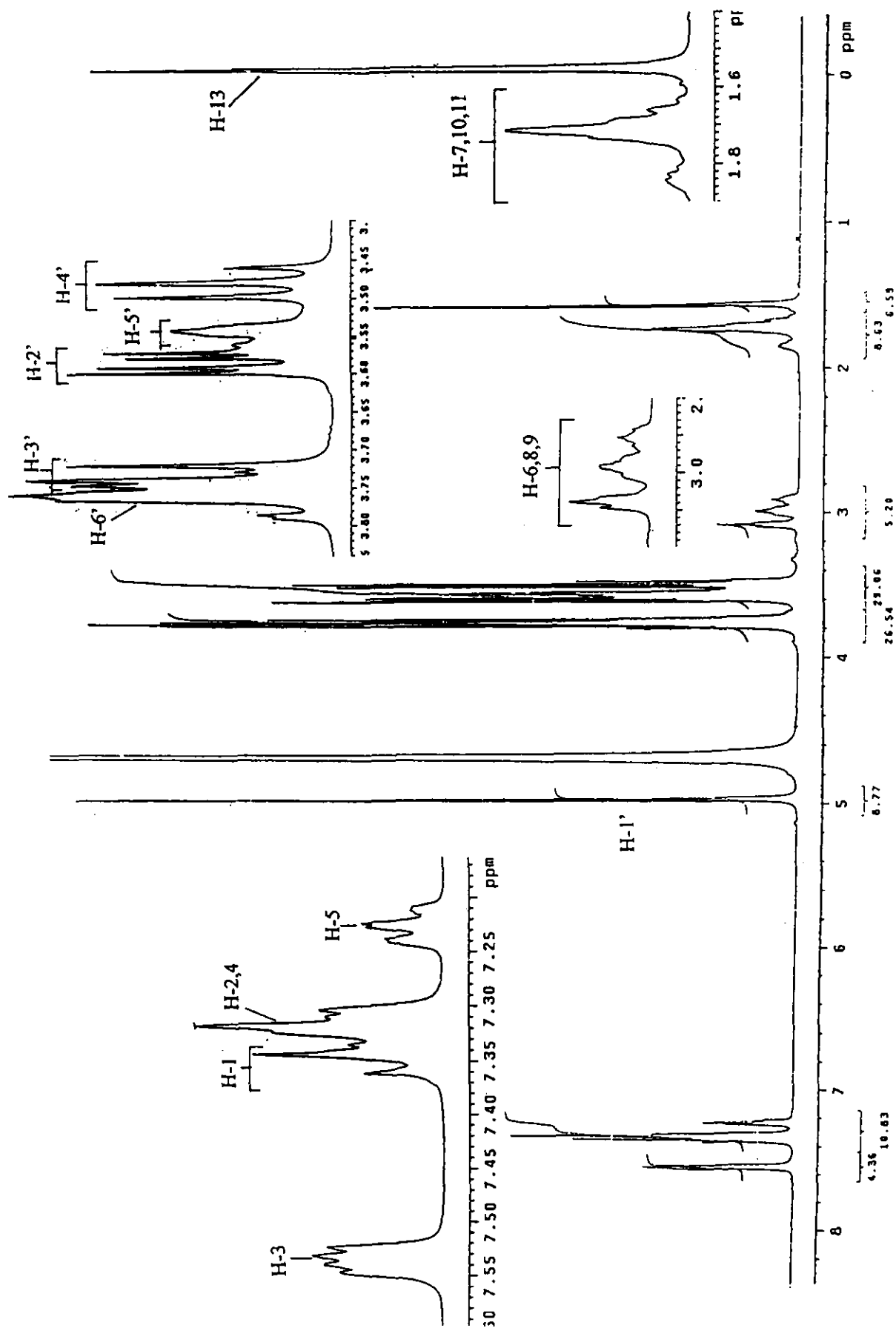


Fig. 80 ^1H NMR spectrum (500 MHz) of a mixture of fexofenadine and β -CD having a molar ratio ($[\beta\text{-CD}]/[\text{FFN}]$) equal to 1.15.

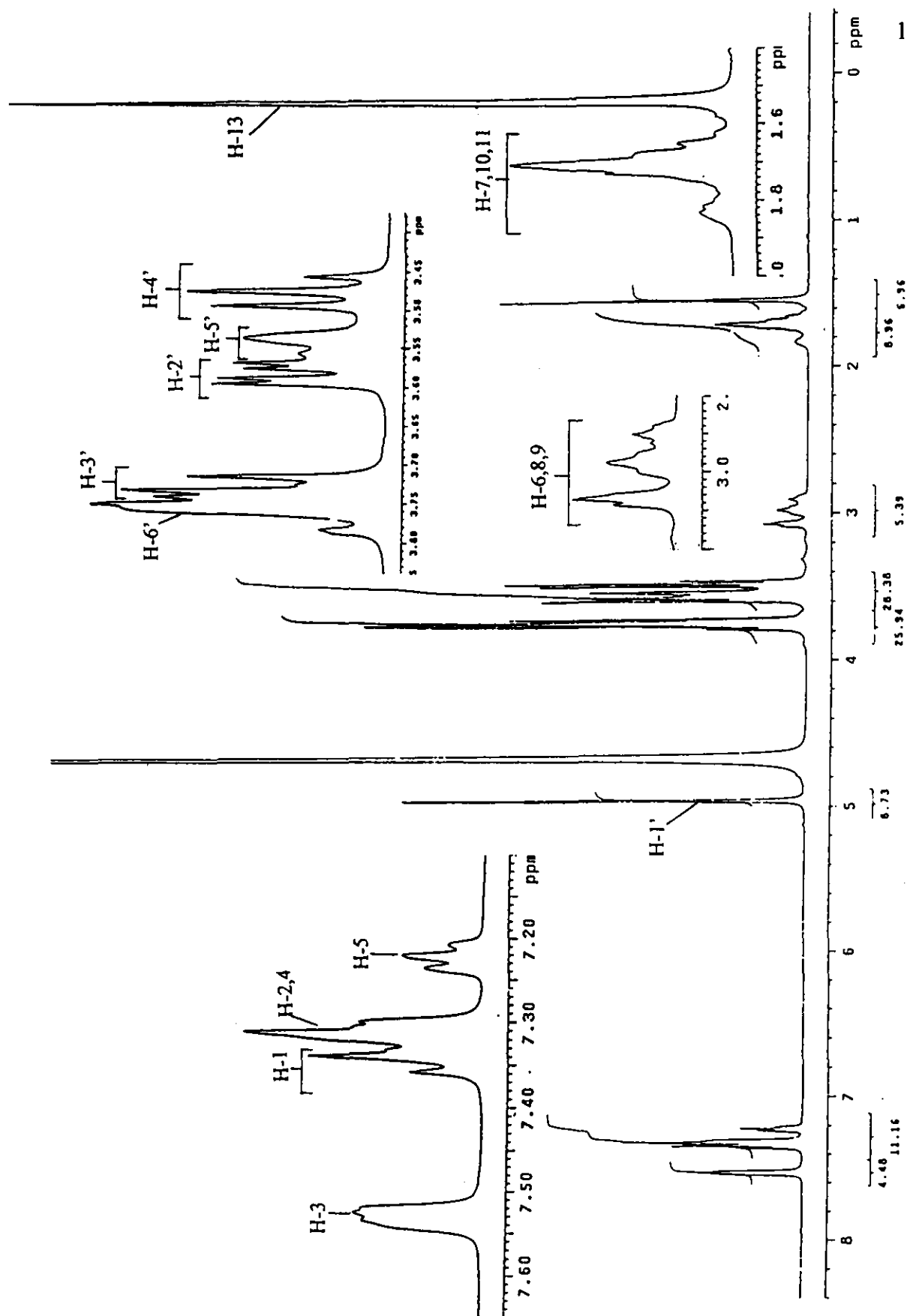


Fig. 81 ^1H NMR spectrum (500 MHz) of a mixture of fexofenadine and β -CD having a molar ratio ($[\beta\text{-CD}]/[\text{FFN}]$) equal to 1.12.

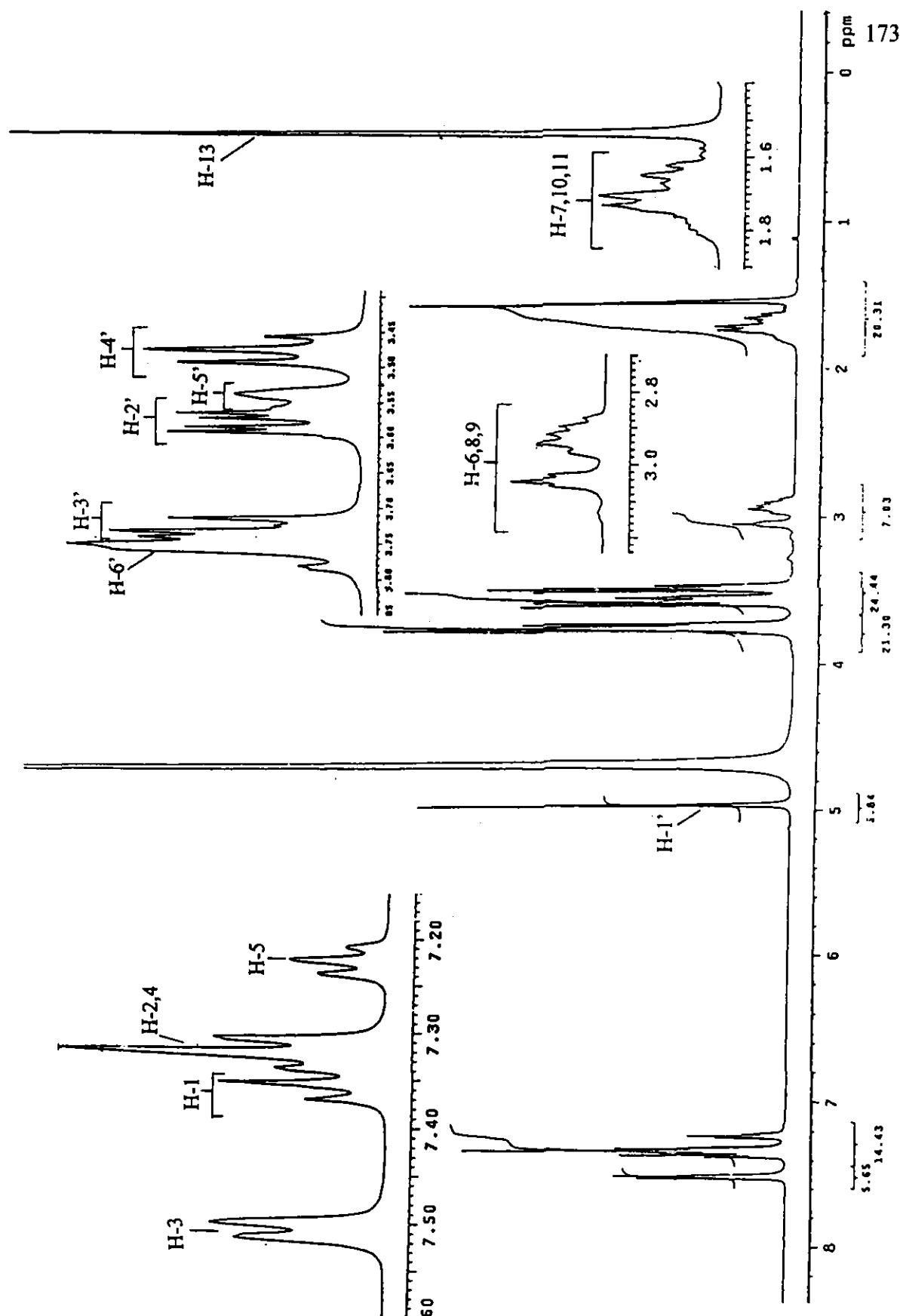


Fig. 82 ^1H NMR spectrum (500 MHz) of a mixture of fexofenadine and β -CD having a molar ratio ($[\beta\text{-CD}]/[\text{FFN}]$) equal to 0.68.

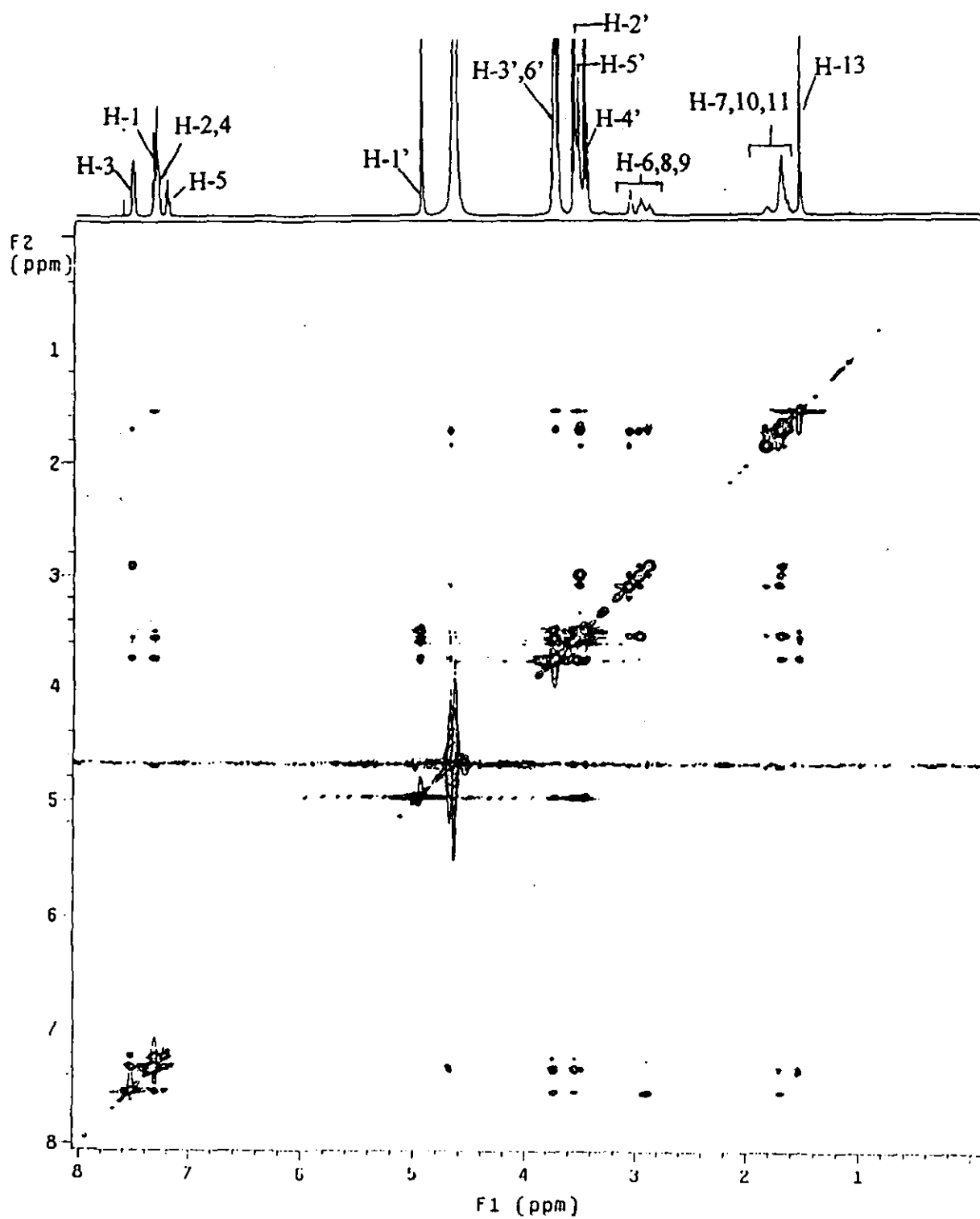


Fig. 83 2D ROESY spectrum (500 MHz) of a mixture of fexofenadine and β -CD.

Result and Discussion

¹H NMR Chemical Shift Change Data of β -CD

All the β -CD proton resonances were easily identified by their characteristic pattern of signals. An examination of the β -CD proton signals in the ¹H NMR spectra revealed significant highfield shift changes in H-3' and H-5' proton resonances, positioned inside the β -CD cavity, compared to pure β -CD. Negligible shift changes were observed in H-2' and H-4' proton signals while small highfield shift changes were observed in H-6' signal. The induced highfield shift changes in the β -CD cavity protons upon addition of FFN confirmed the formation of FFN- β -CD complex in solution in analogy to previous reports.^{233, 237, 261, 262} Fig. 85 shows complexation induced shift changes in the β -CD protons in the presence of FFN compared to pure β -CD.

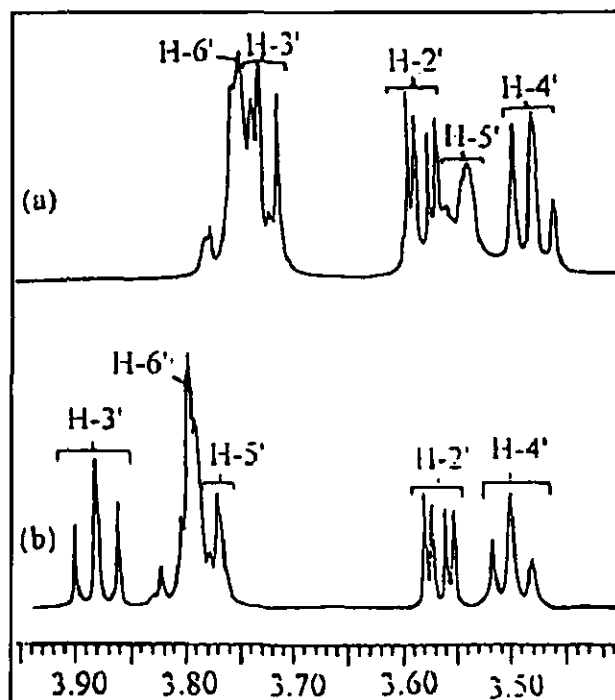


Fig. 85 Part of the ^1H NMR (500 MHz) spectra showing chemical shift changes in the β -CD protons (a) in the presence of FFN in comparison to (b) pure β -CD.

^1H NMR Assignment of Fexofenadine

The assignment of all the FFN protons, especially the aliphatic protons, was not possible because separate signals were not observed for aliphatic protons. It was possible though to make an unambiguous assignment of all the aromatic protons and methyl protons, and therefore only these protons could be studied. The signal for methyl protons (H-13) was observed as a singlet in all the spectra of FFN, pure as well as mixtures of FFN and β -CD. FFN is structurally related to terfenadine,²⁷³ in which the para substituent on ring

A is tertiary butyl group. The ^1H NMR data of terfenadine and ROESY spectral data of FFN, in the presence of β -CD, proved very helpful in the ^1H NMR assignment of FFN. The chemical shift change data for all the studied protons is given in Table 16. The spectral region between 1.498 and 1.809 totally integrated for 14 protons which contained a singlet for six protons assignable to two methyl groups (H-13) while remaining 8 protons could be ascribed to H-7, 10 and 11 protons. Similarly, the region between 2.864 and 3.225 integrating for 5 protons and a signal at 3.4 for two protons, in the spectrum of pure FFN, could be attributed to H-6, 8 (5H) and H-9 (2H) protons, respectively. The signal for H-9 merged with the β -CD protons in the spectra of mixtures. The signal for H-12 can be expected around 4.5 but it was found merged with HDO signal as confirmed by the ROESY spectral data. The signal for H-1 at 7.736 was identified by its through-space interaction with methyl protons (H-13) in the ROESY spectrum (Fig. 84). The signal for H-5, most shielded aromatic proton, was observed as a triplet at 7.227 as in the case of terfenadine. The most deshielded aromatic proton signal at 7.481 has been assigned to H-3 while this signal was ascribed to H-4 in the case of terfenadine. Our assignment is justified because this signal shows cross connection peaks, in the ROESY spectrum, with the signals at 2.909 and 1.714 which are attributable to H-6 and H-7 protons. These NOE peaks can not be explained if the signal at 7.481 is assigned to H-4. The proton resonances for H-2 and H-4 were found merged in the region 7.317-7.348. The signal for H-2 showed a cross peak with the signal for H-11 and H-12, which appeared merged with the water signal.

¹H NMR Chemical Shift Change Data of Fexofenadine

As already mentioned chemical shift change data for only aromatic and methyl protons could be obtained. All the aromatic protons and methyl signal exhibited shift changes in the presence of β -CD which increased with the increase in the concentration of β -CD. The magnitude of chemical shifts for H-3 was very high compared to other aromatic protons. The ring A protons exhibited highfield shift changes while downfield shift changes were observed for ring B protons. The changes in the shapes of the signals for H-3 and H-5 suggest that there is some splitting, probably due to chiral differentiation between the two enantiomers by the β -CD. Part of the ¹H NMR spectra, containing aromatic signals, of FFN in the absence as well as presence of β -CD are depicted in Fig. 86 while chemical shift change data of all the studied protons is given in Table 16.

Table 16 ¹H NMR chemical shift change data of studied protons of FFN in the presence of β -CD. (Temperature = 25 °C, Solvent = D₂O)

[β -CD]/[FFN]	H-1	H-2, 4	H-3	H-5	CH ₃ -13
2.46	-0.060	-0.023	0.109	-0.003	0.068
1.76	-0.058	-0.028	0.089	-0.004	0.038
1.27	-0.042	-0.014	0.060	0.001	0.053
1.15	-0.040	-0.011	0.052	0.000	0.052
1.12	-0.044	-0.017	0.047	-0.005	0.039
0.68	-0.030	-0.010	0.026	-0.004	0.030

Negative values indicate highfield shift changes

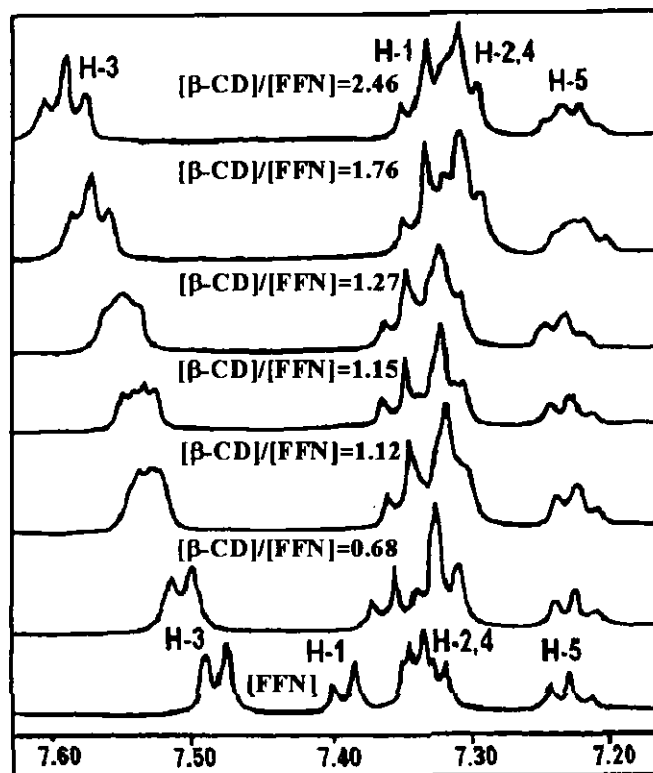


Fig. 86 A part of ^1H NMR spectrum (500 MHz) showing aromatic protons of FFN in the absence as well as in the presence of varying amounts of β -CD. (Temperature = 25 $^\circ\text{C}$, Solvent = D_2O)

Stoichiometry of the FFN- β -CD Complex

Scott plot²⁵² of $\Delta\delta_{\text{H-3}}$ against $[\beta\text{-CD}]$ in the form of $[\beta\text{-CD}]/\Delta\delta_{\text{H-3}}$ verses $[\beta\text{-CD}]$ also gave a nonlinear relationship indicating that the stoichiometry of the complex is not 1:1. The stoichiometry of the complex was then determined by mole ratio method.²⁴⁶ Fig. 87 illustrates plot of $\Delta\delta$ verses mole ratio ($[\beta\text{-CD}]/[\text{FFN}]$) for H-3 of rings B because it showed maximum shift changes in the presence of β -CD. The plot shows that the $\Delta\delta$ values increase in a linear fashion till $[\beta\text{-CD}]/[\text{FFN}]=1$ suggesting 1:1 stoichiometry

but since $\Delta\delta$ does not become constant and still keeps increasing even when $[\beta\text{-CD}]/[\text{FFN}] > 1$ implies that the stoichiometry is not simply 1:1 but a combination of 1:1 and 1:2 (FFN/ β -CD) complexes. The formation of 1:2 complex is favoured when $[\beta\text{-CD}]/[\text{FFN}] > 1$. It was further confirmed by Scott method that the stoichiometry is neither simply 1:1 nor 1:2 but a combination of both.

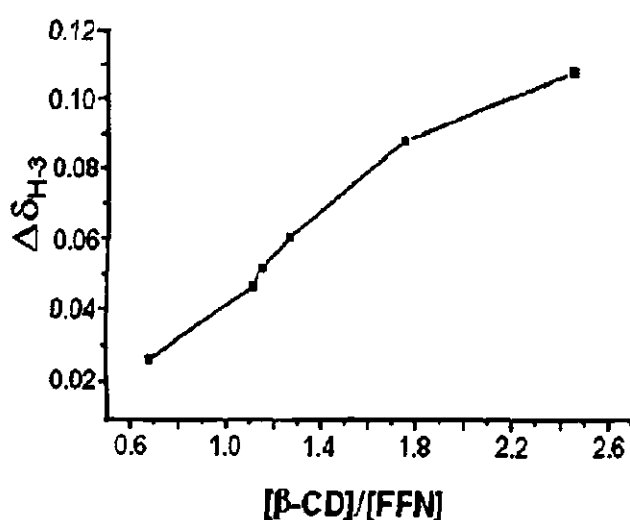


Fig. 87 Mole ratio diagram of the chemical shift changes of FFN proton during titration with β -CD in D_2O .

ROESY Data of FFN- β -CD Mixture

ROESY spectrum was recorded for a mixture of FFN and β -CD having molar ratio equal to 1.15 in D_2O to ascertain the existence of multiple equilibria in solution and to have an insight into the mode of penetration and orientation of the aromatic ring into the β -CD

cavity. As shown in the Fig. 88, the ROESY spectrum displayed correlation peaks between all the aromatic protons with H-3' and H-5' of β -CD situated inside the cavity thus confirming beyond doubt the involvement of all the aromatic rings in the complexation.

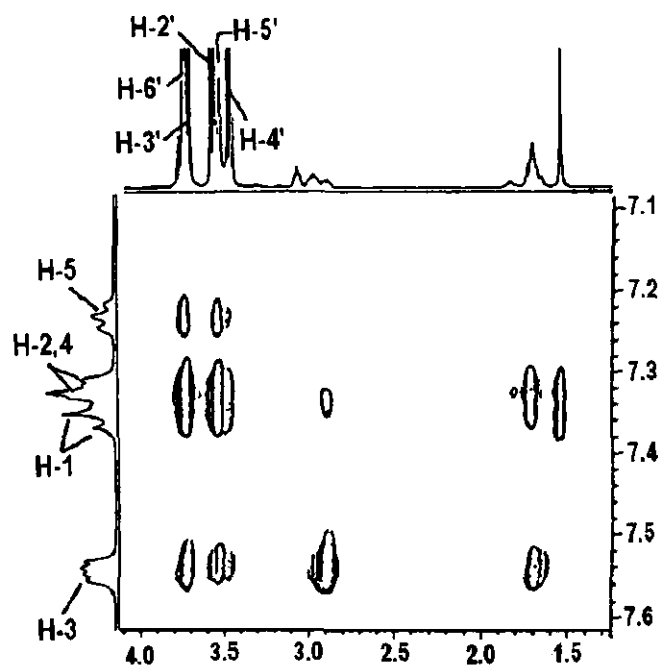


Fig. 88 Expansion of the ROESY spectrum (500 MHz) showing cross peaks of methyl and all the three aromatic ring protons with β -CD protons. (Mixing time = 0.2 sec, Delay Time = 3.05 sec)

The cross peaks between H-3 of FFN and H-2', 4' of β -CD, situated near wider rim clearly indicate that phenyl ring penetrates the cavity from wider rim side while p-substituted ring penetrates from narrower rim side since H-1 is showing cross peak with H-2', 4' of β -CD. Fig. 89 shows the proposed structures for all the possible FFN- β -CD inclusion complexes present in solution.

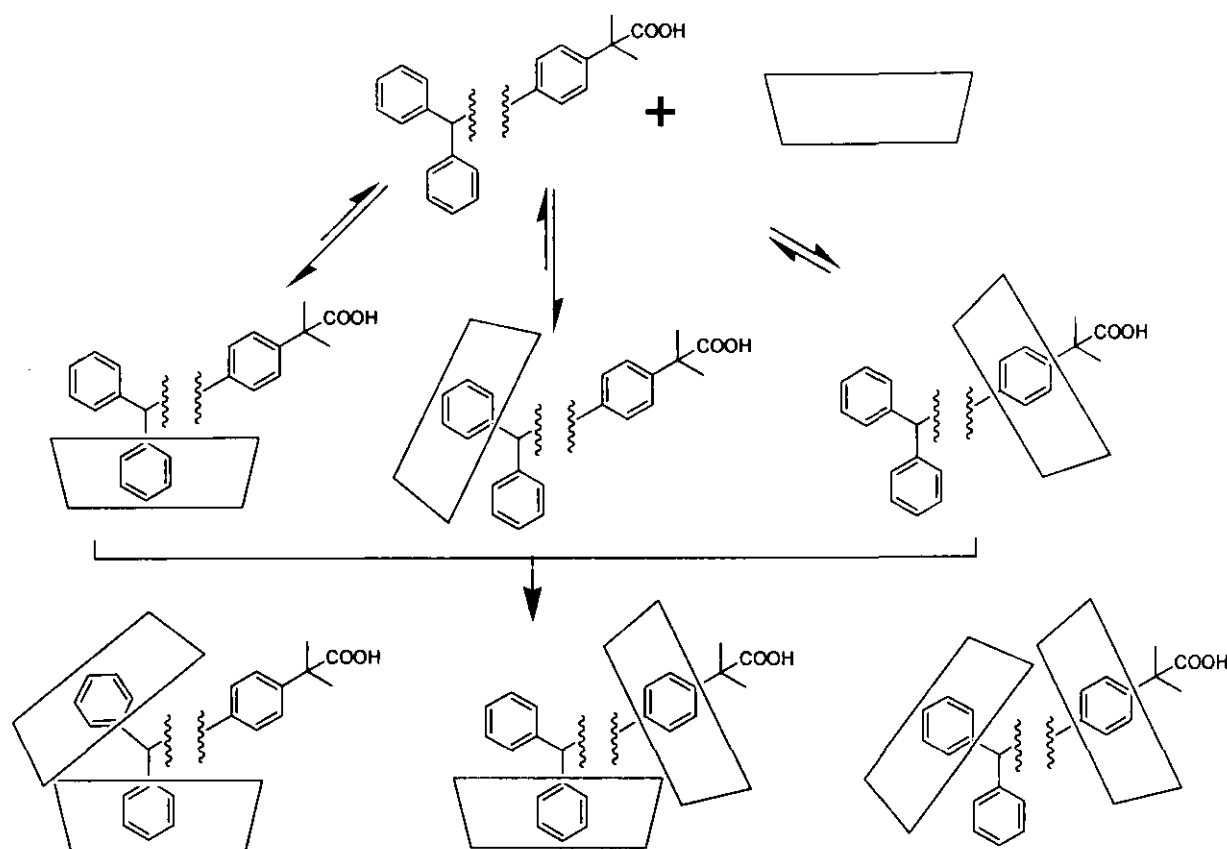


Fig. 89 Schematic representation of all the possible inclusion complexes formed in D₂O between FFN and β-CD.

In conclusion, ¹H NMR titration studies of fexofenadine hydrochloride (FFN) with β-cyclodextrin (β-CD) in D₂O confirmed the presence of several inclusion complexes involving all the three aromatic rings. The structures for all the possible 1:1 and 1:2 complexes have been proposed taking into account the stoichiometry and ROESY spectral data. The mode of penetration has been clearly established. The stability constant could not be calculated but the complexation behaviour of fexofenadine seems very similar to that of terfenadine.²⁷³

Annexure

vii) Conclusion and Suggestion for Further Work

In conclusion, the complexation of five pharmaceutical compounds with β -cyclodextrin was studied, in aqueous solution at room temperature, by NMR spectroscopy which is one of the most acceptable techniques for the study of inclusion complexes in solution. The objective of our work was to establish the structure of the complex formed in solution. It was found, in all the cases, that aromatic part of the drug molecule preferentially enters the β -cyclodextrin cavity resulting in the formation of inclusion complex/es. Moreover, the aromatic ring generally enters the cyclodextrin cavity from wider rim side but complexes involving entry of the guest from narrower rim have also been identified. The mode and depth of the penetration have been established, in an unambiguous manner, by advanced NMR techniques like ROESY and NOESY experiments. The mode and depth of penetration are supported by binding constants. The complexes identified were, in most case, 1:1 (Drug: β -CD) but in the case of enalapril maleate the complex was found to be 2:1 (Drug: β -CD). Fexofenadine forms both 1:1 and 1:2 (drug: β -CD) complexes in aqueous solution.

It can be concluded that, in the cases where only one aromatic ring is present in the drug, 1:1 complex is formed when the guest/host ratio is equal or smaller than one and as the concentration of guest further increases, the 2:1 (drug: β -CD) complex starts to appear and increases with the increase in the concentration of drug. In the cases where more than one aromatic ring was present in the drug molecule, more than one 1:1 complexes were formed.

Furthermore, the penetration of the aromatic ring was shallow when the aromatic ring was meta-disubstituted resulting in the formation of weak complex as evidenced by smaller value of stability constant (K_a) in the case of roxatidine acetate hydrochloride where two bulky groups are present on the aromatic ring in the meta position. The presence of a substituent in the para position may affect the stability of the complex in a favourable or unfavourable manner depending on the nature and size of the substituent.

The significance of the work carried out lies in the fact that use of a pharmaceutical compound in the form of a cyclodextrin complex is permitted only after the structures of the complexes formed, in aqueous solution, are established because they have to work in aqueous medium. The effect of size of the cyclodextrin cavity on the complexation behaviour will be studied by studying the complexation of drugs with α - and γ -CD and with other modified CDs to increase the solubility of drug in water. Further, efforts will be made to do X-ray studies on the purified drug-cyclodextrin complexes.

References

1. A. Villiers, *Compt. Rend.*, **1891**, 112, 536.
2. F. Z. Schardinger, *Unters. Nahr. u. Genussm.*, **1903**, 6, 865.
3. K. Freudenberg, G. Blomquist, L. Ewald, K. Soff, *Ber. Dtsch. Chem. Ges.*, **1936**, 69, 1258.
4. P. Karrer, C. Nageli, *Helv. Chim. Acta.*, **1921**, 4, 169.
5. A. Miekeley, *Ber. Dtsch. Chem. Ges.*, **1932**, 65, 69.
6. D. French, *Adv. Carbohydr. Chem.*, **1957**, 12, 189.
7. F. Cramer, *Einschlussverbindungen (Inclusion Compounds)*, Springer-Verlag: Berlin, **1954**.
8. K. Freudenberg, F. Cramer, H. Plieninger, *Ger. Patent* 895, **1953**, 769.
9. J. Szejtli, Ed.; *Proc. 1st Int. Symp. On Cyclodextrins*, Budapest, **1981**.
10. W. Saenger, *Angew. Chem. Int. Ed. Engl.*, **1980**, 19, 344.
11. J. Szejtli, *Cyclodextrins and Their inclusion Complexes*, Akademiai Kiado: Budapest, **1982**.
12. J. Szejtli, *Cyclodextrin Technology*, Kluwer Academic Publishers: Dordrecht, **1988**.
13. R. Breslow, *Acc. Chem. Res.*, **1995**, 28, 146.
14. R. Breslow, M. F. Czarniecki, J. Emert, H. Hamaguchi, *J. Am. Chem. Soc.*, **1980**, 102, 762.
15. S. Shirin, E. Buncel, G. W. vanLoon, *Can. J. Chem.*, **2003**, 81, 45.
16. A. Konno, M. Misaki, J. Toda, T. Wada, K. Yasumatsu, *Agric. Biol. Chem.*, **1986**, 46, 2203.

17. H. Suzuki, S. Watano, T. Sasazuka, T. Tsutsumi, *U. S. Patent*, **1994**, 5, 314, 877.
18. J. W. Simpkins, *J. Parenter. Sci. Technol.*, **1991**, 45, 266.
19. R. A. Sanchez, S. Hendler, *U. S. Patent*, **1995**, 5, 409, 908.
20. R. A. Sanchez, S. Hendler, *U. S. Patent*, **1995**, 5, 380, 711.
21. U. Clternesi, M. Sciacchitano, *Cosmet. Toiletries Manuf. World Wide*, **1996**, 96, 37.
22. S. Ruppert, D. Knittel, H.-J. Buschmann, G. Wenz, E. Schollmeyer, *Starch/ Starke*, **1997**, 49, 160.
23. W. Lau, B. M. Shah, *U. S. Patent*, **1994**, 5, 376, 709.
24. W. Shieh, A. Hedges, *U. S. Patent*, **1994**, 5, 371, 209.
25. G. Somogyi, J. Posta, L. Buris, M. Varga, *Pharmazie*, **2006**, 61, 154.
26. K. B. Hicks, R. M. Haines, C. B. S. Tong, G. M. Sapers, Y. Atawy, P. L. Irwin, P. A. Seib, *J. Agric. Food Chem.*, **1996**, 44, 2591.
27. P. E. Shaw, C. W. Wilson, *J. Food Sci.*, **1983**, 48, 646.
28. W. Van Uden, H. Oeij, H. J. Woerdenbag, N. Pras, *Plant Cell Tissue Organ Cult.*, **1993**, 34, 169.
29. T. Bruzzese, G. Mazzi, *U. S. Patent*, **1993**, 5, 189, 149.
30. M. Granger, M. Dupont, H. Ledon, *U. S. Patent*, **1996**, 5, 382, 571.
31. S. Cahll, S. Bulusu, *Magn. Reson. Chem.*, **1993**, 31, 731.
32. R. B. Shah, A. Palamakula, M. A. Khan, *J. Pharm. Sci.*, **2004**, 93, 1070.

33. M. Marzona, R. Carpignano, S. Girelli, M. Dolci, In *Proc. 8th Int. Symp. On Cyclodextrins*, J. Szejtli, L. Szenté, Eds., Kluwer Academic Publishers: Dordrecht, The Netherlands, **1996**, p. 619.
34. T. Trinh, In *Proc. 8th Int. Symp. On Cyclodextrins*, J. Szejtli, L. Szenté, Eds., Kluwer Academic Publishers: Dordrecht, The Netherlands, **1996**, p. 541.
35. C. X. Wang, Sh. L. Chen, *J. Industrial Textiles*, **2005**, 34, 157.
36. T. Trinh, D. Phan, WO 94/225501, **1994**.
37. M. Grigoras, D. G. Conduruta, *J. Incl. Phenom. Macro.*, **2006**, 54, 101.
38. Y. Hirai, Y. Shiraishi, H. Shirai, *Macromol. Rapid. Commun.*, **1996**, 16, 697.
39. W. Putteman, J. Caers, J. Masens, J. Peters, *Abst. Int. Confer. Pharmaceutical Applications of Cyclodextrins Conference*, Lawrence. KS, **1997**.
40. A. H. Al-Marzouqi, I. Shehatta, B. Jobe, A. Dowaidar, *Pharm. Sci.*, **2006**, 95, 292.
41. J. Mielcarek, A. Czernielewska, B. Czarczynska, *J. Incl. Phenom. Mol. Recog.*, **2006**, 54, 17.
42. K. Nakanishi, T. Masukawa, T. Nadai, K. Yoshii, S. Okada, K. Miyajima, *Biol. Pharm. Bull.*, **1997**, 20, 66.
43. J. J. Torres-Labandeira, J. Blanco-Mendez, J. L. Vila-Jato, *S. T. P. Pharm. Sci.*, **1994**, 4, 235.
44. B. W. Muller, U. Brauns, *Int. J. Pharm.*, **1985**, 26, 77.

45. A. Yoshida, M. Yamamoto, T. Irie, F. Hirayama, K. Uekama, *Chem. Pharm. Bull.*, **1989**, 37, 1059.
46. K. Shiotani, K. Uehata, T. Irie, F. Hirayama, K. Uekama, *Chem. Pharm. Bull.*, **1994**, 42, 2332.
47. K. Shiotani, T. Irie, K. Uekama, Y. Ishimaru, *Eur. J. Pharm. Sci.*, **1995**, 3, 139.
48. D. O. Thompson, *CRC Crit. Rev. Ther. Drug. Carrier Syst.*, **1997**, 14, 1.
49. M. Yamamoto, A. Yoshida, F. Hiram, K. Uekama, *Int. J. Pharm.*, **1989**, 49, 163.
50. Y. Okada, M. Tachibana, K. Koizumi, *Chem. Pharm. Bull.*, **1990**, 38, 2047.
51. A. Chitjigakis, C. Donze, A. W. Coleman, P. Cardot, *Anal. Chem.*, **1992**, 64, 1632.
52. A. W. Coleman, M. Munoz, A. Chitjigakis, P. Cardot, *J. Phy. Org. Chem.*, **1993**, 6, 651.
53. J. L. H. Johnson, Y. He, A. Jain, S. H. Yalkowsky, *AAPS PharmSciTech.*, **2006**, 7, Article 18.
54. A. M. Sigurdardottir, T. Loftson, *Int. J. Pharm.*, **1995**, 126, 73.
55. H. Fridriksdottir, T. Loftson, E. Stefansson, *J. Control. Rel.*, **1997**, 44, 95.
56. K. P. R. Chowdary, B. N. Nalluri, *Drug Dev. Ind. Pharm.*, **2000**, 26, 1217.
57. B. P. Vilarnovo, I. P. Lopez, M. E. Lopez, P. S. Pardo, E. Estrada, J. J. T Labandeira, *Eur. J. Pharm. Sci.*, **2001**, 13, 331.
58. N. M. Sanghavi, K. B. Choudhari, R. S. Matharu, L. Viswanathan, *Drug Dev. Ind. Pharm.*, **1993**, 19, 701.

59. H. J. Ahn, K. M. Kim, S. J. Choi, C. K. Kim, *Drug Dev. Ind. Pharm.*, **1997**, *23*, 397.
60. M. D. Dhanaraju, K. S. Kumaran, T. Baskaran, M.S.R. Moorthy, *Drug Dev. Ind. Pharm.*, **1998**, *24*, 583.
61. M. D. Veiga, P. J. Diaz, F. Ahsan, *J. Pharm. Sci.*, **1998**, *87*, 891.
62. G. Becket, L. J. Schep, M. Y. Tan, *Int. J. Pharm.*, **1999**, *179*, 65.
63. J. Lotter, H. M. Krieg, K. Keizer, J. C. Breytenbach, *Drug Dev. Ind. Pharm.*, **1999**, *25*, 879.
64. J. M. Askrabic, D. S. Rajic, L. Tasic, S. Djuric, P. Kasa, K. P. Hodi, *Drug Dev. Ind. Pharm.*, **1997**, *23*, 1123.
65. C. Cavallari, B. Abertini, M. L. G. Rodriguez, L. Rodriguez, *Eur. J. Pharm. Biopharm.*, **2002**, *54*, 65.
66. K. P. R. Chowdary, S. S. Rao, *Ind. J. Pharm. Sci.*, **2001**, *63*, 438.
67. M. K. Ghorab, M. C. Adeyeye, *Pharm. Dev. Technol.*, **2001**, *6*, 305.
68. M. J. Arias, J. R. Moyano, P. Munoz, J. M. Gines, A. Justo, F. Giordano, *Drug Dev. Ind. Pharm.*, **2000**, *26*, 253.
69. K. Uekama, T. Fujinaga, M. Hirayama, M. Otagiri, M. Yamasaki, H. Seo, T. Hashimoto, M. Tsuruoka, *J. Pharm. Sci.*, **1983**, *72*, 1338.
70. J. A. Castillo, J. P. Canales, J. J. Garcia, J. L. Lastres, F. Bolas, J. J. Torrado, *Drug Dev. Ind. Pharm.*, **1999**, *25*, 1241.
71. Y. Nagase, M. Hirata, K. Wada, H. Arima, F. Hirayama, T. Irie, M. Kikuchi, K. Uekama, *Int. J. Pharm.*, **2001**, *229*, 163.
72. T. Loftsson, D. S. Peterson, *Drug Dev. Ind. Pharm.*, **1998**, *24*, 365.
73. R. McCandless, S. H. Yalkowsky, *J. Pharm. Sci.*, **1998**, *87*, 1639.

74. V. Londhe, M. Nagarsenker, *Ind. J. Pharm. Sci.*, **1999**, *61*, 237.
75. G. Trapani, A. Latrofa, M. Franco, M. R. Pataleo, E. Sanna, F. Massa, F. Tuveri, G. Liso, *J. Pharm. Sci.*, **2000**, *89*, 1451.
76. A. Latrofa, G. Trapani, M. Franco, M. Serra, M. Muggironi, F. P. Fanizzi, A. Cutrignelli, G. Liso, *Eur. J. Pharm. Biopharm.*, **2001**, *52*, 65.
77. S. Baboota, M. Dhaliwal, K. Kohli, *AAPS PharmSciTech.*, **2005**, *6*, E83-E90.
78. G. Bettinetti, A. Gazzaniga, P. Mura, F. Giordano, M. Setti, *Drug. Dev. Ind. Pharm.*, **1992**, *18*, 39.
79. J. Kang, V. Kumar, D. Yang, P. R. Chowdhury, R. J. Hohl, *Eur. J. Pharm. Sci.*, **2002**, *15*, 163.
80. A. C. Jain, M. C. Adeyeye, *Ind. J. Pharm.*, **2001**, *212*, 177.
81. L. Zhao, P. Li, S. H. Yalkowsky, *J. Pharm. Sci.*, **1999**, *88*, 967.
82. A. M. Kaukonen, H. Lennernas, J. P. Mannermaa, *J. Pharm. Pharmacol.*, **1998**, *50*, 611.
83. H. Arima, K. Yunomae, K. Miyake, T. Irie, F. Hirayama, K. Uekama, *J. Pharm. Sci.*, **2001**, *90*, 690.
84. G. Bettinetti, P. Mura, M. T. Faucci, M. Sorrenti, M. Setti, *Eur. J. Pharm. Sci.*, **2002**, *15*, 21.
85. J. L. Koontz, J. E. Marcy, W. E. Barbeau, S. E. Duncan, *J. Agric. Food Chem.*, **2003**, *51*, 7111.
86. B. D. Wagner, G. J. McManus, *Anal. Biochem.*, **2003**, *317*, 233.
87. N. O. Brown, D. L. Butler, P. K. Chiang, *J. Pharm. Pharmacol.*, **1993**, *45*, 666.

88. F. J. Konings, M. K. M. Noppe, J. L. Mesens, *U. S. Patent* , **1994**, 5, 376, 632.
89. J. Mielcarek, *Pharmazie*, **1996**, 51, 474.
90. K. Uekama, T. Fujinaga, F. Hirayama, M. Otagiri, Y. Kuroono, K. Ikeda, *J. Pharm. Pharmacol.*, **1982**, 34, 627.
91. F. Hirayama, M. Kurihara, K. Uekama, *Chem. Pharm. Bull.*, **1986**, 34, 5093.
92. H. Adachi, T. Irie, F. Hirayama, K. Uekama, *Chem. Pharm. Bull.* **1992**, 40, 1586.
93. Y. Horiyoshi, F. Hirayama. K. Uekama, *Yakugaku Zasshi*, **1991**, 111, 592.
94. S. Scalia, S. Villani. A. Casolari, *J. Pharm. Pharmacol.*, **1999**, 51, 1367.
95. R. B. Jayachandra, J. K. Pandit, *Drug Dev. Ind. Pharm.*, **1999**, 25, 1215.
96. B. Cwiertnia, T. Hladon, M. Stobiecki, *J. Pharm. Pharmacol.*, **1999**, 51, 1213.
97. J. Li, Y. Guo, G. Zograf, *J. Pharm. Sci.*, **2002**, 91, 229.
98. M. E. Brewster, T. Loftsson, K. S. Estes, J. L. Lin, H. Fririksdoottir, *Int. J Pharm.*, **1992**, 79, 289.
99. D. Q. Ma, R. A. Rajewski, D. V. Velde, V. J. Stella, *J. Pharm Sci.*, **2000**, 8, 275.
100. A. K. Singla, A. Garg, D. Aggarwal, *Int. J. Pharm.*, **2002**, 235, 179.
101. P. Jahro, D. V. Velde, V. J. Stella, *J. Pharm. Sci.*, **2000**, 89, 241.
102. S. Sortino, S. Giuffrida, G. D. Guldi, R. Chillemi, S. Petralia, G. Marconi, G. Condorelli, S. Sciuto, *Photochem. Photobiol*, **2001**, 73, 6.
103. W. Leng, L. Oin, X. Tang, *J. Am. Sci.*, **2006**, 2, 61.

104. Z. Wang, Y. Deng, S. Sun, X. Zhang, *Drug Dev. Ind. Pharm.*, **2006**, 32, 73.
105. S. Jambhekar, R. Casella, T. Maher, *Int. J. Pharm.*, **2004**, 270, 149.
106. T. Tokunaga, M. Nambu, Y. Tsushima, K. Tatsuishi, M. Kayano, Y. Machida, T. Nagai, *J. Pharm. Sci.*, **1986**, 75, 391.
107. R. A. Rajewski, V. J. Stella, *J. Pharm. Sci.*, **1996**, 85, 1142.
108. T. Loftsson, M. E. Brewster, M. Masson, *Am. J. Drug Deliv.*, **2004**, 2, 261.
109. H. Seo, K. Uekama, *Yakugaku Zasshi*, **1989**, 109, 778.
110. G. T. Taylor, J. Weuss, J. Pitha, *J. Pharm. Res.*, **1989**, 6, 641.
111. C. A. Stuenkel, R. E. Dudley, S. S. C. Yen, *J. Clin. Endocrinol. Metabol.*, **1991**, 72, 1054.
112. K. Uekama, T. Imai, T. Irie, F. Hirayama, M. Otagiri, *J. Pharm. Sci.*, **1985**, 74, 841.
113. M. Kikuchi, F. Hirayama, K. Uekama, *Int. J. Pharm.*, **1987**, 38, 191.
114. H. Arima, T. Kondo, T. Irie, F. Hirayama, K. Uekama, T. Miyaji, Y. Inoue, *Yakugaku Zasshi*, **1982**, 112, 65.
115. H. Arima, T. Irie, K. Uekama, *Int. J. Pharm.*, **1989**, 57, 107.
116. K. Uekama, T. Kondo, K. Nakamura, T. Irie, K. Arakawa, M. Shibuya, J. Tanaka, *J. Pharm. Sci.*, **1995**, 84, 15.
117. S. Wang, D. Li, Y. Ito, X. Liu, J. Zhang, C. Wu, *J. Pharm. Pharmacol.*, **2004**, 56, 1251.
118. A. Usayapant, A. H. Karara, M. M. Narurkar, *Pharm. Res.*, **1991**, 8, 1495.

119. P. Jarho, A. Urtta, D. W. Pate, P. Suhonen, T. Jarvinen, *Int. J. Phar.*, **1996**, *137*, 209.
120. J. K. Kristinsson, H. Fridriksdottir, S. Thorisdottir, A. M. Sigurdardottir, E. Stefansson, T. Loftsson, *Ophthalmol. Visual Sci.*, **1996**, *37*, 1199.
121. H. Fridriksdottir, T. Loftsson, E. Stefansson, *J. Control. Rel.*, **1997**, *44*, 95.
122. H. H. Sigurosson, E. Stefansson, E. Guomundsdottir, T. Eysteinnsson, M. Thorsteinsdottir, T. Loftsson, *J. Control. Rel.*, **2005**, *102*, 255.
123. S. Nicoli, P. Santi, *J. Control. Rel.*, **2006**, *111*, 89.
124. K. Uekama, K. Arimori, A. Sakai, K. Masaki, T. Irie, M. Otagiri, *Chem. Pharm. Bull.*, **1987**, *35*, 2910.
125. H. Arima, H. Adachi, T. Irie, K. Uekama, J. Pitha, *Pharm. Res.*, **1990**, *7*, 1152.
126. H. Arima, T. Miyaji, T. Irie, F. Hirayama, K. Uekama, *Chem. Pharm. Bull.*, **1996**, *44*, 582.
127. D. H. Correa, P. S. Melo, C. A. de Carvalho, M. B. de Azevedo, N. Duran, M. Haun, *Eur. J. Pharmacol.*, **2005**, *510*, 17.
128. P. Sukonen, T. Jarvinen, K. Lehmussaari, K. Reunamiski, A. Urtti, *Pharm. Res.*, **1995**, *12*, 529.
129. O. Reer, T. K. Bock, B. W. Muller, *J. Pharm. Sci.*, **1994**, *83*, 1345.
130. T. Irie, M. Otagiri, K. Uekama, Y. Okano, T. Miyata, *J. Incl. Phenom. Macro.*, **1984**, *2*, 637.
131. H. Arima, T. Kondo, T. Irie, K. Uekama, *J. Pharm. Sci.*, **1992**, *81*, 1119.
132. J. H. Perrin, F. P. Field, D. A. Hansen, R. A. Mufson, G. Torosian, *Res. Commun. Chem. Pathol. Pharmacol.*, **1978**, *19*, 372.

133. J. Pitha, S. Zawadzki, F. Chytil, D. Lotan, R. Lotan, *J. Natl. Cancer Inst.*, **1980**, *65*, 1011.
134. J. Pitha, *J. Bioact. Compatible Polym.*, **1988**, *3*, 157.
135. J. Pitha, L. Szente, *Life Sci.*, **1983**, *32*, 719.
136. T. O. Carpenter, J. M. Pettifor, R. M. Russell, J. Pitha, S. Mobarhan, M. S. Ossip, S. Wainer, *C. S. Anaest, J. Paediatr.*, **1987**, *111*, 507.
137. K. Uekama, k. Shiotani, T. Irie, Y. Ishimaru, J. Pitha, *J. Pharm. Pharmacol.*, **1993**, *45*, 745.
138. C. J. Betlach, M. A. Gonzalez, B. C. Kckiernan, C. Neff-Davis, N. Bodor, *J. Pharm. Sci.*, **1993**, *82*, 1058.
139. Y. Horiuchi, F. Hirayama, K. Uekama, *J. Pharm. Sci.*, **1990**, *79*, 128.
140. F. Hirayama, N. Hirashima, K. Abe, K. Uekama, T. Ijitstu, M. Ueno, *J. Pharm. Sci.*, **1988**, *77*, 233.
141. K. Uekama, Y. Horiuchi, T. Irie, F. Hirayama, *Carbohydr. Res.*, **1989**, *192*, 323.
142. A. Sayani, Y. W. Chien, *CRC Crit. Rev. Ther. Drug Carrier Syst.*, **1996**, *13*, 85.
143. K. Uekama, *Yakugaku Zasshi*, **2004**, *124*, 909.
144. K. Wada, H. Arima, T. Tsutsumi, F. Hirayama, K. Uekama, *Biol. Pharm. Bull.*, **2005**, *28*, 500.
145. S. H. Pun, N. C. Bellocq, A. Liu, G. Jensen, T. Machemer, E. Quijano, T. Schluep, S. Wen, H. Engler, J. Heidel, M. E. Davis, *Bioconjug. Chem.*, **2004**, *15*, 831.
146. E. Pop, T. Loftsson, N. Bodor, *Pharm. Res.*, **1991**, *8*, 1044.

147. G.C. Cotzias, P. S. Papavasiliou, R. Gellene, *N. Eng. J. Med.*, **1969**, 280, 337.
148. H. K. Kroemer, J. Turgeon, R. A. Parker, D. M. Roden, *Clin. Pharmacol. Ther.*, **1989**, 46, 584.
149. D. E. Drayer, *Clin. Pharmacol. Ther.*, **1986**, 40, 125.
150. P. H. Vlasses, Irvin, P. B. Huber, R. B. Lee, R. K. Ferguson, J. J. Schrogie, A. G. Zacchei, R. O. Davies, W. B. Abrams, *Clin. Pharmacol. Ther.*, **1981**, 29, 798.
151. J. J. Baldwin, W. B. Abrams, In *Drug Stereochemistry-Analytical Methods and Pharmacology*, Ed. I. W. Wainer, D. E. Drayer, Marcel Dekker, **1988**, p. 311.
152. G. Gubitz, M. G. Schmid, *Mol. Biol.*, **2006**, 32, 159.
153. D. W. Armstrong, T. J. Ward, R. D. Armstrong, T. E. Beesley, *Science*, **1986**, 232, 1134
154. K. Mislow, M. Raban, *Topu Stereochem.*, **1967**, 1, 1.
155. A. Laverde, G. J. A. Jr. da Conceicao, S. C. N. Queiroz, F. Y. Fujiwara, A. J. Marsaioli, *Magn. Reson. Chem.*, **2002**, 40, 433.
156. A. Botsi, B. Perly, E. Hadjoudis, *J. Chem. Soc. Perkin Trans 2*, **1997**, 89.
157. H. Dodziuk, W. Kozminski, O. Lukin, D. Sybilska, *J. Mol. Struct.*, **2000**, 523, 205.
158. R. Dyllick-Brenzinger, J. D. Roberts, *J. Am. Chem. Soc.*, **1980**, 102, 1166.
159. A. Taylor, C. A. Biackledge, J. K. Nicholson, D. A. R. Williams, I. D. Wilson, *Anal. Proc.*, **1992**, 29, 229.
160. K. Kano, Y. Kato, M. Kodera, *J. Chem. Soc. Perkin Trans 2*, **1996**, 1211.

161. Y. Liu, B. Li, T. Wada, Y. Inoue, *Tetrahedron*, **2001**, 57, 7153.
162. H. Dodziuk, J. Sitkowski, L. Stefaniak, D. Sybilska, *Pol. J. Chem.*, **1996**, 70, 1361.
163. K. B. Lipkowitz, S. Ragothama, J. Yang, *J. Am. Chem. Soc.*, **1992**, 114, 1554.
164. D. D. Traficante, D. M. In Grant, R. K. Harris editors, *Encyclopedia of Nuclear Magnetic Resonance.*, Chichester, Wiley, **2002**, Vol. 6, p. 3988.
165. D. Canet, J. B. Robert, J. J. Delpuech, *Dynamics of Solutions and Fluid Mixtures by NMR*, Chichester, Wiley, **1995**, p. 19.
166. T. Kitae, T. Nakayama, K. Kano, *J. Chem. Soc. Perkin Trans 2*, **1998**, 207.
167. T. Kitae, H. Takashima, K. Kano, *J. Incl. Phenom Macro.*, **1999**, 33, 345.
168. S. K. Branch, U. Holzgrabe, T. M. Jefferies, H. Malwitz, M. W. Matchet, *J. Pharm. Biomed. Anal.*, **1994**, 12, 1507.
169. B. Chankvetadze, N. Burjanadze, G. Pintore, D. Strickmann, D. Bergenthal, G. Blaschke, *Chirality*, **1999**, 11, 635.
170. K. Kano, Y. Tamiya, C. Otsuki, T. Shimomura, T. Ohna, O. Hayashida, Y. Murakami, *Supramol. Chem.*, **1993**, 2, 137.
171. Y. Yamashoji, T. Ariga, S. Asano, M. Tanaka, *Anal. Chim. Acta*, **1992**, 268, 39.
172. B. Chankvetadze, N. Burjanadze, K. D. Maynard, K. Bergander, D. Bergenthal, G. Blaschke, *Electrophoresis*, **2002**, 23, 3027.
173. B. Chankvetadze, I. Kartoziya, N. Burjanadze, D. Bergenthal, H. Luftmann, G. Blaschke, *Chromatographia*, **2001**, 53, S290.
174. S. Negi, K. Terai, K. Kano, K. Nakamura, *J. Chem. Res. (S)*, **1998**, 750.

175. B. Chankvetadze, *Electrophoresis*, **2002**, 23, 4022.
176. B. Chankvetadze, N. Burjanadze, J. Breitkreutz, J. Breitkreutz, K. Bergander, D. Bergenthal, O. Kataeva, R. Frohlich, H. Luftmann, G. Blaschke, *J. Sep. Sci.*, **2002**, 25, 1155.
177. B. Chankvetadze, G. Pintore, N. Burjanadze, D. Bergenthal, K. Bergander, J. Breitkreutz, C. Muhlenbrock, G. Blaschke, *J. Chromatogr. A*, **2000**, 875, 455.
178. B. Chankvetadze, K. Lomsadze, N. Burjanadze, J. Breitkreutz, G. Pintore, M. Chessa, K. Bergander, G. Blaschke, *Electrophoresis*, **2003**, 24, 1083.
179. B. Chankvetadze, M. Fillet, N. Burjanadze, D. Bergenthal, K. Bergander, H. Luftmann, J. Crommen, G. Blaschke, *Enantiomer*, **2000**, 5, 313.
180. B. Chankvetadze, N. Burjanadze, G. Pintore, D. Bergenthal, K. Bergander, C. Muhlenbrock, J. Breitkreutz, G. Blaschke, *J. Chromatogr. A*, **2000**, 875, 471.
181. J. L. Atwood, J. E. D. Davies, D. D. MacNicol, *Inclusion Compounds*, Academic: London, **1984**.
182. A. K. Yatsimirskii, A. V. Eliseev, *J. Chem. Soc. Perkin Trans. 2*, **1991**, 1769.
183. M. Suzuki, K. Ito, C. Fushimi, T. Kondo, *Chem. Pharm. Bull.*, **1993**, 41, 942.
184. M. Kitagawa, H. Hoshi, M. Sakurai, Y. Inoue, R. Chujo, *Carbohydr. Res.*, **1987**, C1, 163.
185. J. Kohler, M. Hohla, R. Sollner, M. Amann, *Supramol. Chem.*, **1998**, 5, 117.
186. M. Kitagawa, H. Hoshi, M. Sakurai, Y. Inoue, R. Chujo, *Bull. Chem. Soc. Jpn.*, **1988**, 61, 4225.

187. M. Sakurai, M. Katagawa, H. Hoshi, Y. Inoue, R. Chujo, *Carbohydr. Res.*, **1990**, *198*, 181.
188. K. A. Connors, S. F. Lin, A. B. Wong, *J. Pharm. Sci.*, **1982**, *71*, 217.
189. D. M. Davies, J. R. Savage, *J. Chem. Res. (S)*, **1993**, 94.
190. K. Kano, T. Ishimura, S. Negi, *J. Incl. Phenom. Macro.*, **1995**, *22*, 285.
191. D. M. Davies, M. E. Deary, D. I. Wealleans, *J. Chem. Soc. Perkin Trans. 2*, **1998**, 193.
192. S. Hamai, N. Satoh, *Carbohydr. Res.*, **1997**, *304*, 229.
193. S. Yasuda, K. Miyake, J. Sumaoka, M. Komiyama, H. Shigekawa, *Jpn. J. Appl. Phys.*, **1999**, *38*, 3888.
194. E. Junquera, E. Aicart, *J. Pharm. Sci.*, **1999**, *88*, 626.
195. M. R. Eftink, M. L. Andy, K. Bystrom, H. D. Perimutter, D. S. Kristol, *J. Am. Chem. Soc.*, **1989**, *111*, 6765.
196. M. Suzuki, H. Takai, H. Ohmori, *Supramol. Chem.*, **1994**, *3*, 133.
197. J. S. Menka, D. S. Lawrence, *Tetrahedron Lett.*, **1989**, *30*, 7341.
198. A. Gafai, Y. Cohen, *J. Org. Chem.*, **1997**, *62*, 120.
199. K. B. Lipkowitz, *Chem. Rev.*, **1998**, *98*, 1829.
200. P. Mu, M. Fujie, Y. Matsui, *Bull. Chem. Soc. Jpn.*, **1993**, *66*, 2084.
201. H. S. Frank, M. W. Evans, *J. Chem. Phys.*, **1945**, *13*, 507.
202. J. L. Finney, A. K. Soper, *Chem. Soc. Rev.*, **1994**, *23*, 1.
203. M. Komiyama, M. L. Bender, *J. Am. Chem. Soc.*, **1978**, *100*, 2259.
204. R. I. Gelb, L. M. Schwartz, D. A. Laufer, *Bioorg. Chem.*, **1980**, *9*, 450.

205. I. Gomez-Orellana, D. Hallen, M. Stoodeman, *J. Chem. Soc. Faraday Trans.* **1994**, *90*, 3397.
206. N. Matsuura, S. Takenaka, N. Tokura, *J. Chem. Soc. Perkin Trans. 2*, **1977**, 1419.
207. R. Rymden, J. Carlfors, P. Stilbs, *J. Incl. Phenom. Macro.* **1983**, *1*, 159.
208. O. S. Tee, C. Mazza, R. Lazano-Hemmer, *J. Org. Chem.*, **1994**, *59*, 7602.
209. G. C. Kresheck, H. Schneider, H. A. Scheraga, *J. Phys. Chem.*, **1965**, *69*, 3132.
210. W. Saenger, T. Steiner, *Acta Cryst.*, **1998**, *A54*, 798.
211. T. Steiner, W. Saenger, *J. Am. Chem. Soc.*, **1992**, *114*, 10146.
212. T. Aree, J. Jacob, W. Saenger, H. Hoier, *Carbohydr. Res.*, **1998**, *307*, 191.
213. T. Steiner, W. Saenger, *J. Chem. Soc. Chem. Commun.*, **1995**, 2087.
214. S. P. van Helden, B. P. van Eijck, L. H. M. Janssen, *J. Biomol. Struct. Dyn.*, **1992**, *9*, 1269.
215. M. Shibakami, S. Sekiya, *J. Chem. Soc. Chem. Commun.*, **1992**, 1742.
216. I. L. Alderfer, A. V. Eliseev, *J. Org. Chem.*, **1997**, *62*, 8225.
217. K. Kano, M. Tasumi, S. Hashimoto, *J. Org. Chem.*, **1991**, *56*, 6579.
218. S. I. Takahashi, E. I. Suzuka, N. Nagashima, *Bull. Chem. Soc. Jpn.*, **1986**, *59*, 1129.
219. Z. P. Yi, H. L. Chen, Z. Z. Huang, Q. Huang, J. S. Yu, *J. Chem. Soc. Perkin Trans. 2*, **2000**, 121.
220. B. Hingerty, W. Saenger, *J. Am. Chem. Soc.*, **1976**, *98*, 3357.
221. M. R. Eftink, J. C. Harrison, *Bioorg. Chem.*, **1981**, *10*, 388.

- 222. D. W. Griffiths, M. L. Bender, *Adv. Catal.*, **1973**, 23, 209.
- 223. S. Takagi, M. Fujisawa, T. Kimura, *Thermochim. Acta*, **1991**, 183, 289.
- 224. C. J. Bender, *Chem. Soc. Rev.*, **1986**, 15, 1686.
- 225. J. T. Rademacher, A. W. Czarnik, *J. Am. Chem. Soc.*, **1993**, 115, 3018.
- 226. K. Kano, K. Mori, B. Uno, M. Goto, T. Kubota, *J. Am. Chem. Soc.*, **1990**, 112, 8645.
- 227. L. Liu, X. S. Li, T. W. Mu, Q. X. Guo, Y. C. Liu, *J. Incl. Phenom. Macro.*, **2000**, 38, 199.
- 228. K. S. Song, L. Liu, X. S. Li, Q. X. Guo, *Res. Chem. Intermed.*, **2000**, 26, 319.
- 229. C. Anselmi, M. Centini, M. Ricci, A. Buonocore, P. Granata, T. Tsuno, R. M. Facino, *J. Pharm. Biomed. Anal.*, **2006**, 40, 875.
- 230. G. Piel, S. Moutard, B. Perly, S. H. de Hassonville, P. Bertholet, V. Barillaro, M. Piette, L. Delattre, B. Evrard, *J. Drug Deliv. Sci.*, **2004**, 14, 91.
- 231. Y. Ikeda, F. Hirayama, H. Arima, K. Uekama, Y. Yoshitake, K. Harano, *J. Pharm. Sci.*, **2004**, 93, 1659.
- 232. W. R. Croasmun, R. M. K. Carlson, *Two-Dimensional NMR-spectroscopy*, VCH: New York, **1994**.
- 233. P. V. Demarco, A. L. Thakkar, *J. Chem. Soc. Chem. Commun.*, **1970**, 2.
- 234. Y. Liu, L. Li, H. Y. Zhang, Z. Fan, X. D. Guan, *Biorg. Chem.*, **2003**, 31, 11.
- 235. M. Bogdan, M. R. Caira, D. Bogdan, C. Morai, S. I. Farcas, *J. Incl. Phenom. Macro.*, **2004**, 49, 225.

- 236. E. Bednarek, W. Bocian, J. Poznanski, J. Sitkowski, N. S. Sosnowska, L. Kozerski, *J. Chem. Soc. Perkin Trans. 2*, **2002**, 999.
- 237. H.-J. Schneider, F. Hacket, V. Rudiger, H. Ikeda, *Chem. Rev.*, **1998**, 98, 1755.
- 238. D. Salvatiera, C. Jaime, A. Virgili F. Sanchez-Ferando, *J. Org. Chem.*, **1996**, 61, 9578.
- 239. E. Estrada, I. P. Lopez, J. J. Torres-Labandeira, *J. Org. Chem.*, **2000**, 65, 8510.
- 240. M. V. Rekharsky, R. N. Goldberg, F. P. Schwarz, Y. B. Tewari, P. D. Ross, Y. Yamashoji, Y. Inoue, *J. Am. Chem. Soc.*, **1995**, 117, 8830.
- 241. T. Nakajima, M. Sunagawa, T. Hirohashi, K. Fujioka, *Chem. Pharm. Bull.*, **1984**, 32, 383.
- 242. K. Kano, H. Hasegawa, *J. Am. Chem. Soc.*, **2001**, 123, 10616.
- 243. G. Fronza, A. Mele, E. Redenti, P. Ventura, *J. Pharm. Sci.*, **1992**, 81, 1162.
- 244. R. Bergeron, R. Rowan 111, *Biorg. Chem.*, **1976**, 5, 425.
- 245. D. Neuhaus, M. P. Williamson, *The Nuclear Overhauser Effect*, VCH: Weinheim, **1989**.
- 246. V. Rudiger, A. Eliseev, S. Simova, H.-J. Schneider, M. J. Blandamer, P. M. Cullis, A. J. Meyer, *J. Chem. Soc. Perkin Trans. 2*, **1996**, 2119.
- 247. D. Salvatiera, C. Jaime, A. Virgili, F. Sanchez-Ferando, *J. Org. Chem.*, **1996**, 61, 9578.
- 248. K. Kano, H. Kano, S. Negi, T. Kitae, R. Takaoka, M. Yamaguchi, H. Okubo, M. Hirama, *J. Chem. Soc. Perkin Trans. 2*, **1999**, 15.
- 249. P. Job, *Ann. Chem.*, **1928**, 9, 113.

- 250. K. Connors, *Binding Constant: The Measurement of Molecular Complex Stability*, Wiley: New York, **1987**.
- 251. H. A. Benesi, J. H. Hildebrand, *J. Am. Chem. Soc.*, **1949**, *71*, 2703.
- 252. R. Mathur, E. D. Becker, R. B. Bradley, N. C. Li, *J. Phys. Chem.*, **1963**, *67*, 2190.
- 253. M. W. Hanna, A. L. Ashbaugh, *J. Phys. Chem.*, **1964**, *68*, 811.
- 254. R. Foster, C. A. Fyfe, *J. Chem. Soc. Faraday Trans.*, **1965**, *61*, 1626.
- 255. G. Scatchard, *Ann. N. Y. Acad. Sci.*, **1949**, *51*, 660.
- 256. R. L. Scott, *Rev. Trav. Chim. Pays-Bas.*, **1956**, *75*, 787.
- 257. W. Liu, B. Tang, Y. Wang, Z. Chen, *Anal. Lett.*, **2004**, *37*, 755.
- 258. G. M. Escangar, *Analyst*, **1999**, *124*, 587.
- 259. F. R. Schulze, A. Buschauer, W. Schunack, *Eur. J. Pharm. Sci.*, **1998**, *6*, 177.
- 260. J. D. Collin, A. W. Pidgen, *Drugs*, **1988**, *35*, 41.
- 261. K. Eliadou, K. Yannakopoulou, A. Rontoyianni, I. M. Mavridis, *J. Org. Chem.*, **1999**, *64*, 6217.
- 262. K. S. Cameron, D. Fietcher, L. Fielding, *Magn. Reson. Chem.*, **2002**, *40*, 251.
- 263. B. G. Pollock, *Exp. Opin. Pharmacother.*, **2001**, *2*, 681.
- 264. M. J. Owens, D. L. Knight, C. B. Nemeroff, *Biol. Psychiatry*, **2001**, *50*, 345.
- 265. J. G. Hardmann, L. E. Limbird, P. B. Molinoff, R. W. Ruddon, A. G. Gilman, *Eds. Goodman & Gilman's the Pharmacological Basis of Therapeutics*, McGraw Hill: New York, **1996**.

- 266. A. A. W. Elzainy, X. Gu, F. E. R. Simons, K. J. Simons, *AAPS Pharm. Sci.*, **2003**, 5, Article 28.
- 267. J. D. Cleary, J. W. Taylor, *Drug Intell. Clin. Pharm.*, **1986**, 20, 177.
- 268. P. A. Todd, R. C. Heel, *Drugs*, **1986**, 31, 198.
- 269. V. Slavinska, D. Sile, G. I. Chipens, Y. Balodis, G. Rozenenthal, K. Venteris, E. Lukevics, *Chem. Heterocyc. Comp.*; **2003**, 39, 1579.
- 270. G. Fronza, A. Mele, E. Redente, P. Ventura, *J. Org. Chem.*, **1996**, 61, 909.
- 271. M. J. Wyvratt, E. W. Tristram, T. J. Ikeler, N. S. Lohr, H. Joshua, J. P. Springer, B. H. Arison, A. A. Patchett, *J. Org. Chem.*, **1984**, 49, 2816.
- 272. K. Simpson, B. Jarvis, *Drugs* **2000**, 59, 301.
- 273. E. Redenti, M. Pasini, P. Ventura, A. Spisni, M. Vikmon, J. Szejtli, *J. Incl. Phenom. Mol. Recogn. Chem.*, **1993**, 15, 281.

LIST OF PUBLICATIONS

1. Complexation of Roxatidine Acetate Hydrochloride with β -Cyclodextrin: NMR Spectroscopic study, *Pharmazie*, **2004**, 59, 653.
2. A Proton Magnetic Resonance Study of Fexofenadine/ β -Cyclodextrin Inclusion Complexes in Aqueous Solution, *Bull. Korean Chem. Soc.*, **2005**, 26, 2061.
3. Complexation of Enalapril Maleate with β -Cyclodextrin: NMR Spectroscopic Study in Solution, *Quimica Nova*, **2006 (In Press)**
4. NMR Spectroscopy of Inclusion Complex of D-(-)-Chloramphenicol with β -Cyclodextrin in Aqueous Solution, *IL Farmaco*, **2004**, 59, 835.
5. Complexation of Fluoxetine Hydrochloride with β -Cyclodextrin. A Proton Magnetic Resonance Study in Aqueous Solution, *IL Farmaco*, **2005**, 60, 445.
6. Complexation of Fluvastatin Sodium with β -Cyclodextrin: NMR Spectroscopic Study in Solution, *J. Incl. Phenom. Macro.*, **2006 (In Press)**
7. High Resolution NMR Spectroscopic Study of Complexation of Hydroxyzine Hydrochloride with β -Cyclodextrin in Aqueous Solution, *J. Chin. Chem. Soc.* **(Communicated)**
8. ^1H NMR Spectroscopic Study of Complexation of Citalopram with β -Cyclodextrin in Aqueous Solution, *Bull. Chem. Soc. Jpn.* **(Communicated)**

30 min) (Pharmakopea Polska 1993). It is interesting that an increasing proportion of xylitol in model tablets is accompanied by an increase in measurable tablet diameter during storage. Average values of disintegration time of model tablets are specified in Table 2. This property, resulting from so called "crystallographic memory" of the xylitol structure, described by the approximation equation, should be taken into consideration during design of packaging (blisters) not only to reduce losses during blistering but also to provide the required practical stability of a preparation.

Experimental

1. Excipients

Sorbitol: Neosorb P 60 W (Roquette-Lestrem, France); Xylitol: Xylisorb 300 (Roquette-Lestrem, France); Hydroxypropylmethyl cellulose (HPMC): Pharmacoat 904 (France); Dibasic calcium phosphate - $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$ (Calcium ortho phosphate p.a.) P.OCH Gliwice (Poland), C-72-04 Budenheim; Magnesium stearate

2. Instruments and methods

"EXACTA 21" numerical tableting machine with computer control which enables monitoring of the compression process at programmed morphological parameters of a model tablet. "TURBULA type T2C" mixer with standard glass containers $V = 3.0 \text{ dm}^3$ in which tablet feed (powder) for direct tableting was made. Electronic micrometer produced by Mitutoyo (U.K.) Ltd; Patent EP 0053091. Measurement of morphological values of a tablet ($d = 2r$; h) was made with an accuracy of $\pm 0.01 \text{ mm}$. Hardness tester: Schleuniger Type T 2 C (Switzerland) and ERWEKA type TB - M (Germany). ERWEKA instrument to determine tablet disintegration time.

The product for direct tableting was prepared in a TURBULA T 2C mixer by mixing Neosorb P 60W, Xylisorb 300, HPMC and $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$ for 15 min. The comparative particle size of the mixed components was maintained in a formulation. After introducing the magnesium stearate the components were mixed in alternating planes for 5 min at a speed of 20 rpm. The compression process was carried out in an "EXACTA 21" tableting machine using computer optimization of the morphological parameters of model tablets.

The different types of model tablets were produced in quantities of 4.5–5.0 thousand units.

Tests were made according to the requirements of FP V (Pharmakopea Polska 1993). The results obtained for each of the four model types, which are characterized by different contents of xylitol, were estimated statistically.

The evaluation of hardness of the model tablets was made with a Schleuniger T 2 C hardness tester *ex tempore* and after the first (12 months) and second (24 months) year of storage. The measurements were made for 20 tablets selected at random and were analysed statistically.

References

- Pharmakopea Polska (1993) Wydawnictwo Polskiego Towarzystwa Farmaceutycznego V, t II, Warszawa p. 65. (Polish Pharmacopoeia).
- Garr JSM (1990) Direct compression characteristic of xylitol. *Int J Pharm* 64: 223–226.
- Goczko H, Szabo-Reveas P, Pasztor-Turak M, Pintye-Hodi K, Eros I (2001) Polyols in the development of chewable tablets containing magnesium aspartate. *Pharm Ind* 63: 639–643.
- Molokhia AM, Moustafa MA, Gouda MW (1982) Effect of storage conditions on the hardness, disintegration and drug release from some tablets bases. *Drug Dev Ind Pharm* 8: 283–292.
- Schmidt PC, Vortisch V (1987) Influence of manufacturing method of fillers and binders their tableting properties comparison of 8 commercially available sorbitols *Pharm Ind* 49: 495–503.
- Wade A, Weller JP (1994) Handbook of Pharmaceutical Excipients, Second Edition, American Pharmaceutical Association, Washington, The Pharmaceutical Press, London.

Department of Chemistry, Aligarh Muslim University, Aligarh, India

Complexation of roxatidine acetate hydrochloride with β -cyclodextrin: NMR spectroscopic study

S. M. ALI, A. MAHESHWARI, F. ASMAT

Received October 13, 2003, accepted January 6, 2004

Dr. Syed Mashhood Ali, Department of Chemistry, Aligarh Muslim University, Aligarh – 202 002 (U.P.), India
smashhoodali@yahoo.com

Pharmazie 59: 653–655 (2004)

A NMR spectroscopic study of mixtures of varying ratios of roxatidine acetate hydrochloride (RAH) and β -cyclodextrin (β -CD) in D_2O revealed the formation of a 1:1 inclusion compound. The aromatic ring of RAH selectively penetrates the β -CD cavity in preference to the piperidine ring.

Cyclodextrins (CDs) hold a variety of guests into their hydrophobic cavities (Steed and Atwood 2000; Wimmer et al. 2002) and serve as useful models for studying topochemistry and catalytic mechanism of enzymes (Bender 1988). CDs are widely used in pharmaceutical applications as a vehicle for drug delivery of poorly water-soluble drugs (Aithal and Shrinivas 1996; Frömming and Szejtli 1994). Extensive studies on inclusion complexes of various medicinally useful compounds with β -CD have been reported (Aithal and Shrinivas 1996; Frömming and Szejtli 1994; Menard et al. 1990). We report, herein, our results on the study of the inclusion complex of roxatidine acetate hydrochloride (RAH), a potent anti-ulcer drug (Murdoch 1991), with β -CD in solution by NMR spectroscopy.

The ^1H NMR spectra were recorded for five samples with [RAH]/[β -CD] molar ratios varying from 0.77 to 4.55 as determined by direct integration of the NMR signals. The anomeric proton (H-1) of β -CD was used as internal reference throughout this work.

The signals for RAH and β -CD protons did not interfere with each other in any spectrum. The signals for aromatic protons of the RAH molecule appeared as a singlet (H-6'), a doublet ($J = 8.1 \text{ Hz}$ H-2',4') and a triplet ($J = 8.0 \text{ Hz}$ H-3') in all the cases except in the spectrum of the pure drug in which the doublet for H-2',4' and H-6' singlet were found partly overlapping.

The induced chemical shift change ($\Delta\delta$) for β -CD protons were calculated relative to reported ^1H NMR data (Schneider et al. 1998). Each sample spectrum denoted largest $\Delta\delta$ for the H-3 and H-5 protons of the β -CD. The other β -CD's protons showed either much smaller or negligible deviations. The $\Delta\delta$ data for various protons of β -CD in the presence of varying amount of RAH is given in the Table. In the presence of RAH, the peaks for H-3 and H-5 protons of the β -CD moved progressively upfield with the increase of concentration of RAH. The Fig. shows the part of the NMR spectra of samples A to E, containing signals for protons of β -CD. NMR spectra of samples A and B were recorded on a 200 MHz spectrometer while those of samples C, D & E were obtained on a 300 MHz instrument. The spectra of samples A and B

Table: ^1H NMR chemical shift changes ($\Delta\delta_i$) of β -CD and RAH protons upon complexation

Sample	[G]/[H]	β -Cyclodextrin						Roxatidine acetate hydrochloride		
		H-2	H-3	H-4	H-5	H-6	$\Delta\delta_i$ (H-5)/ $\Delta\delta_i$ (H-3)	H-2',4'	H-3'	H-6'
A	0.77	0.0141	-0.0176	0.0093	-0.0182	-0.0075	1.03	0.0214	0.0302	-0.0388
B	1.19	0.0107	-0.0276	0.0079	-0.0281	-0.0095	1.01	0.0155	0.0220	-0.0322
C	1.96	0.0060	-0.0495	0.0095	-0.0505	-0.0135	1.02	0.0100	0.0120	-0.0130
D	3.50	0.0140	-0.0985	0.0145	-0.0995	-0.0135	1.01	0.0070	0.0082	-0.0087
E	4.55	0.0170	-0.1455	0.0185	-0.1535	-0.0255	1.05	0.0050	0.0041	-0.0037

Negative values indicates upfield shift.

are very much alike due to small change in [RAH]/[β -CD] molar ratio but the shift of signals for H-3 and H-5 protons is obvious. The shift of signals for H-3 and H-5 protons in the spectra C, D and E is very prominent.

The penetration of the less polar portion of the guest molecule into the β -CD cavity from the wider side comprises the most common mode of complexation. Since the H-3 and H-5 protons are positioned inside the cavity, the inclusion of guest into the β -CD cavity causes major upfield shifts of these protons. This is attributed to the ring current of the aromatic ring of the guest molecule that is included in the cavity (Komiyama and Hirai 1980a, 1980b). On the other hand, most of the guest protons show downfield shift changes but the $\Delta\delta_i$ values for guest protons are much smaller in magnitude compared to host protons. Rekharsky et al. (1995) have demonstrated that the magnitude of the upfield shifts of H-3 and H-5 protons of CDs, $\Delta\delta_i(\text{H-3})$ and $\Delta\delta_i(\text{H-5})$, and their relative ratios $\Delta\delta_i(\text{H-5})/\Delta\delta_i(\text{H-3})$, can be used, respectively, as a quantitative measure of the stability of complex and the depth of inclusion of ligand into the cavity.

The observed high field shifts in the H-3 and H-5 peaks of β -CD in the presence of varying amount of RAH thus

clearly indicate the insertion of the aromatic ring of the RAH into the β -CD cavity. Moreover, the magnitude of the shifts for H-3 and H-5 protons of β -CD increased as a function of an increasing ratio of [RAH]/[β -CD] giving a slightly curved line for $\Delta\delta_i(\text{H-3})$ vs [RAH]/[β -CD] plot. A modified Hildebrand-Benesi plot (Benesi and Hildebrand 1949; Qi et al. 1991) of chemical shift change data for H-3 of β -CD in the form of ([RAH]/[β -CD])/ $\Delta\delta_i(\text{H-3})$ vs [RAH]/[β -CD] gave excellent linear fits supporting the 1:1 complex formation. As expected, all the protons of the RAH, except the aromatic proton (H-6'), showed downfield shifts on complexation. The $\Delta\delta_i$ data for aromatic protons of RAH in the presence of varying amounts of β -CD is given in the Table. There was no significant change observed in the chemical shift values of protons of RAH molecule in the spectra of samples C, D and E. This is in good agreement with the earlier observation that with the increase of concentration of guest, the $\Delta\delta_i$ values for guest protons decrease (Rekharsky et al. 1995). This phenomenon indicated, in analogy to many previously reported cases (Bergeron and Rowan 1976; Komiyama and Hirai 1980a, 1980b), that the phenyl ring of the RAH is selectively inserted into the β -CD cavity driven primarily by hydrophobic interactions.

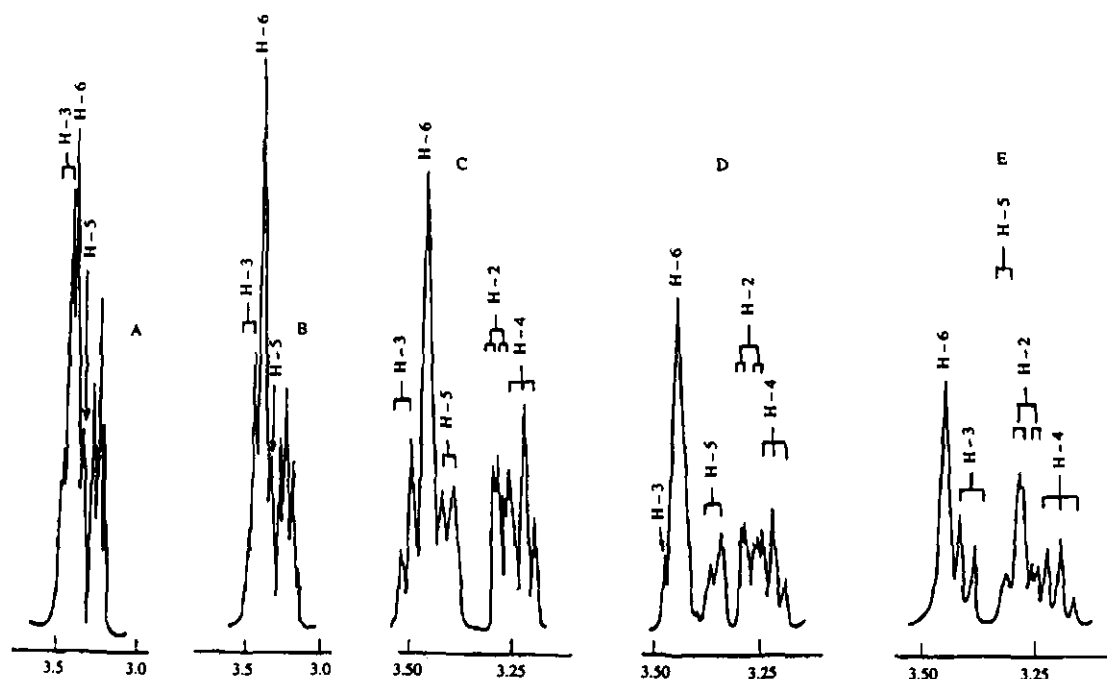


Fig.: Typical ^1H NMR spectra at a variety of guest to host [G]/[H] ratios for the complexation of Roxatidine Acetate Hydrochloride (RAH) with β -cyclodextrin in D_2O . Note the large upfield shifts of H-3 and H-5 in sharp contrast to the small shift changes observed for H-2, H-4 and H-6

The $\Delta\delta_i$ values for H-2 and H-4 of β -CD and H-6' aromatic proton of RAH need special attention. Normally the signal for all the β -CD protons show upfield shifts (if any) while all the guest protons show downfield shifts. The downfield shifts of H-2 and H-4 of β -CD and upfield shift of H-6' of RAH may be attributed to their interaction through solvent. The ratios $\Delta\delta_i(\text{H-5})/\Delta\delta_i(\text{H-3})$ were calculated for all the samples. The relatively smaller values (1.01–1.05) for $\Delta\delta_i(\text{H-5})/\Delta\delta_i(\text{H-3})$ ratios compared to those reported for other complexes (1.2–3.0) suggest that the penetration of the aromatic ring in the β -CD cavity is not deep which is expected due to the presence of two bulky groups in the meta position of aromatic ring. Finally, the ^1H NMR spectroscopic study of RAH in the presence of β -CD shows that a 1:1 RAH- β -CD complex is formed in solution which is in rapid equilibrium with free β -CD and RAH since the spectra consisted of mainly one set of resonances. Moreover, the aromatic ring of the RAH selectively penetrates into the β -CD cavity driven by hydrophobic interactions

References

- Aithal K, Shrinivas UN (1996) Physicochemical study of ciprofloxacin with β -CD. *Pharma Sci* 2: 451–455.
- Bender ML (1988) *Enzyme Mechanisms*. Royal Society of Chemistry, Letchworth, p. 1.
- Benesi HA, Hildebrand JH (1949) Interaction of I with aromatic hydrocarbon. *J Am Chem Soc* 71: 2703–2707.
- Bergeron RJ, Rowan R (1976) The molecular disposition of sodium p-nitrophenolate in the cavities of cycloheptaamylose and cyclohexaamylose in solution. *Bioorg. Chem.* 5: 423–436.
- Frömming K, Szejtli J (1994) *Cyclodextrin in pharmacy*. Kluwer Academic Dordrecht, p. 1.
- Komiyama M, Hirai H (1980a) Time averaged conformation of the inclusion complexes of β -cyclodextrin with tert-butylphenols. *Chem Lett* 12: 1467–1470.
- Komiyama M, Hirai H (1980b) Relationship between the cyclodextrin catalysis in the cleavages of phenyl acetates and the time averaged conformation of the inclusion complexes. *Chem Lett* 12: 1471–1474.
- Menard FA, Dedhiya MG, Rhodes CT (1990) Physicochemical aspects of the complexation of some drugs with cyclodextrins. *Drug Dev Ind Pharm* 16: 91–113.
- Murdoch D (1991) Roxatidine acetate: a review of its pharmacodynamic and pharmacokinetic properties and its therapeutic potential in peptic ulcer disease and related disorder. *Drugs* 42: 240–60.
- Qi ZH, Mak V, Diaz L, Grant DM, Chang CJ (1991) Molecular Recognition: α -cyclodextrin and penicillin V inclusion complexation. *J Org Chem* 56: 1537–1542.
- Rekharasy MV, Goldberg RN, Schwarz FP, Tiwari YB, Ross PD, Yamashoji Y, Inoue Y (1995) Thermodynamic and Nuclear Magnetic Resonance study of the interactions of α - and β -cyclodextrin with model substances. Phenylethylamine, ephedrine and related substances. *J Am Chem Soc* 117: 8830–8840.
- Schneider HJ, Hackett F, Rudiger V (1998) NMR studies of cyclodextrins and cyclodextrin complexes. *Chem Rev* 98: 1755–1785.
- Steed JW, Atwood JL (2000) *Supramolecular Chemistry*. Wiley-VCH, Weinheim, p. 1.
- Wimmer R, Aachmann FL, Larsen KL, Petersen SB (2002) NMR diffusion as a novel tool for measuring the association constant between cyclodextrin. *Carbohydr Res* 337: 841–849.
- Guangdong Key Laboratory of Marine Materia Medica, South China Sea Institute of Oceanology, Chinese Academy of Sciences, Guangzhou, P.R. China

A new triterpenoid: taraxerol-3- β -O-tridecyl ether from *Derris trifoliata*

LU-RONG XU, SI ZHANG, JUN WU, SHU-HUA QI, JIAN-SHE HUANG, ZHI-HUI XIAO, DAO-JING ZHANG, JIN YANG, YAN TIAN

Received January 12, 2004, accepted February 20, 2004

Si Zhang, Guangdong Key Laboratory of Marine Material Medica, South China Sea Institute of Oceanology, Chinese Academy of Sciences, 164 West Xingang Road, Guangzhou, 510301, P.R. China.
zhsimd@scsio.ac.cn

Pharmazie 59: 655–656 (2004)

The new triterpenoid taraxerol-3- β -O-tridecyl ether was isolated from the aerial part of *Derris trifoliata*. The structure was established on the basis of spectral data.

The genus *Derris* (Leguminous family) is widely spread in southeast Asia. It is employed for pest control in horticulture, agriculture and in poultry (Gupta et al. 1999). Some species are also used in folk medicine (Sekine et al. 1999; Mahidol et al. 1997). *D. trifoliata*, a woody climber growing in the coastal forest throughout south east Asia, is used for poisoning fish by local people. The whole plant is also used as a stimulant, antispasmodic, and counter-irritant (Ramachandran et al. 1986). Previous investigations of leaves of *Derris trifoliata* have yielded hydrocarbons, wax esters (Misra et al. 1987), sterols, amyryl, lupeol (Ghosh et al. 1985), and two flavonol glycosides: rhamnetin-3-O- β -neohesperidoside and quercetin-3-O- β -neohesperidoside (Ramachandran et al. 1986). We have investigated the chemical constituents of the aerial parts of *D. trifoliata*, and report here the isolation and characterization of a new etherified triterpenoid.

Compound 1, prisms crystalline (CHCl_3 - CH_3OH), was formulated as $\text{C}_{43}\text{H}_{76}\text{O}$ from ESI-MS (m/z 647 $[\text{M} + \text{k}]^+$, 631 $[\text{M} + \text{Na}]^+$). Its ^1H and ^{13}C NMR spectra showed great similarity to that of taraxerol (Sakurai et al. 1987). In ^1H NMR, eight tertiary methyls at δ 1.09 (3H, s), 0.98 (3H, s), 0.95 (3H, s), 0.93 (3H, s), 0.91 (6H, s), 0.82 (3H, s) and 0.80 (3H, s) were typical for triterpene; an oxymethine proton at δ 3.20 (1H, dd, $J = 11.0, 4.6$ Hz, H-3) was observed; a downfield one proton double doublet centered at δ 5.53 ($J = 11.3, 3.2$ Hz, H-15) indicated the presence of an olefinic bond, and was assigned to the olefinic proton at C-15 of the taraxerane skeleton (Talaputra et al. 1981); Two geminal methylene protons at δ 1.92 (1H, dd, $J = 14.7, 2.9$ Hz, H-16a) and 2.03 (1H, m, H-16b) could be discerned in the ^1H NMR spectra; Chemical shifts for one oxymethine carbon at δ 79.08 (C-3), olefinic carbons at δ 158.10 (C-14) and 116.88 (C-15) were all observed. Thus, the carbocyclic nucleus of compound 1 proved to be that of a typical taraxerol. Additional signals as one oxymethylene carbon at δ 63.11, one terminal methyl carbon at δ 14.11, multiple methylene carbon signals at δ 29.34 ~ 29.70 indicated that the hydroxyl of taraxol was substituted by a long alkane. And

A Proton Magnetic Resonance Study of Fexofenadine/ β -Cyclodextrin Inclusion Complexes in Aqueous Solution

Syed Mashhood Ali* and Arti Maheshwari

Department of Chemistry, Aligarh Muslim University, Aligarh-202002, UP, India. *E-mail: smashhoodali@yahoo.com
Received July 7, 2005

Key Words : Fexofenadine hydrochloride, β -Cyclodextrin, Inclusion complex, ^1H NMR, ROESY

Fexofenadine hydrochloride (FFN), (\pm)-4-[1-hydroxy-4-{4-(hydroxydiphenylmethyl)-1-piperidinyl}-butyl]- α,α -dimethyl benzeneacetic acid hydrochloride, is a second generation antihistamine that is used to treat allergies.¹ It is a racemate and exists as zwitter ion in aqueous media at physiological pH. It belongs to the group of amine compounds bearing diphenylmethyl functionality. Like other members of this group, the drug is highly hydrophobic and slightly soluble in water.

The study of inclusion complexes of cyclodextrins (CDs) is a subject of great interest.²⁻⁴ CDs are oligosaccharides composed of six to eight glucopyranose units bound by $\alpha(1\rightarrow4)$ linkages that are commonly named α -, β -, and γ -CD, respectively. β -CD, in particular, has an internal cavity shaped like a truncated cone. The interior of the cavity is relatively hydrophobic while the outer surface is quite hydrophilic because of the presence of numerous hydroxyl groups. CDs can accommodate a variety of guests into its cavity through non-covalent interactions. These complexes serve as models to mimic enzyme activity⁵ and to provide understanding of the molecular recognition.⁶ Moreover, the physical properties, such as solubility, stability, volatility, sublimation, etc. of the guest molecule are modified upon complexation with CDs, and the resulting inclusion complexes have found numerous practical applications in pharmaceutical sciences and in several other areas of chemistry ranging from analytical to synthetic chemistry.⁴⁻⁶

Inclusion complexes of pharmaceutical compounds with CDs have been extensively studied and utilized to improve the solubility,⁷ dissolution rate⁸ and bioavailability of poorly water-soluble drugs.⁹ Other applications of CD complexes of pharmaceuticals include elimination of undesirable drug properties, such as irritation and unpleasant odor or taste. CDs have also been used to stabilize and protect degradation of unstable compounds. In addition, they have shown a potential for improving the stability of light and oxygen sensitive drugs.¹⁰

Various techniques are used to study the CD inclusion complexes but NMR spectroscopy has been found to be most useful in this type of studies.¹¹ Evidence for the inclusion of the guest into the CD-cavity is obtained by simple NMR titration experiments. NMR spectra of mixtures of CD and guest molecule are recorded and changes in the chemical shifts of both the host and guest are studied. The formation of the inclusion complex results in upfield shift

changes in the CD protons situated inside the cavity, namely H-3' and H-5'. On the other hand, guest protons generally experience downfield shift changes but sometimes upfield shifts are also observed. These shift changes are attributed to the anisotropic ring current effect of the aromatic guests. Moreover, the magnitude of the chemical shift changes for the CD-cavity protons have been shown to be a qualitative measure of the stability of the complex while their ratio, $\Delta\delta_{\text{H-3'}/\Delta\delta_{\text{H-5'}}$, gives information about the depth of penetration.¹² Also, information regarding the mode of penetration of the guest into the cavity, i.e. from narrower or wider rim side, can be obtained from these shift changes. A typical inference is that $\Delta\delta_{\text{H-3'}} > \Delta\delta_{\text{H-5'}}$ if the guest enters the cavity from narrower side and *vice versa*¹³ but there are exceptions and these conclusions can only be drawn when only one complex is formed while in cases where multiple equilibria exist these shift changes can only be used as an evidence for the formation of inclusion complexes. Information regarding the stoichiometry and association and/or dissociation constant of the complex can also be obtained by the treatment of simple ^1H NMR titration data.¹⁴

2D NMR spectroscopy has recently become an important tool for the investigation of the interactions between CDs and guest molecules^{11,15} since the NOE cross peaks between the protons that are closer than 4 Å in space are observed in ROESY spectrum. The relative intensities of these cross peaks depend on the spaces between the corresponding protons. The height and the diameter of the β -CD cavity are about 7.9 ± 0.1 Å and 6.0-6.5 Å, respectively.¹⁶ Therefore, while the guest molecule is included into the β -CD cavity, NOE correlation peaks between the protons of the guest and protons of the β -CD cavity (H-3' and H-5') are observed by means of ROESY experiment. According to the relative intensities of these cross peaks, it is possible to estimate the orientation of the guest molecule within the CD cavity.

In continuation of our work on the NMR studies of inclusion complexes of pharmaceutical compounds with β -CD,^{17,18} we report herein our results on the detailed study of the complexation between fexofenadine hydrochloride (FFN) and β -CD in aqueous solution using high resolution NMR spectroscopy.

Results and Discussion

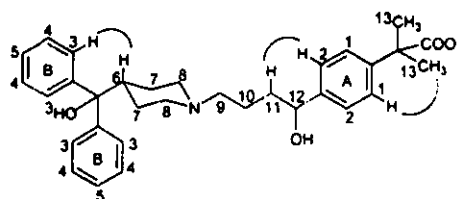
All the NMR spectra were recorded on an Inova 500

instrument in D₂O at 25 °C. The chemical shift values, reported in δ (ppm), were calculated with reference to HDO peak at 4.76. The concentration of the guest was kept constant at 3.7×10^{-3} M while the concentration of the β -CD was varied from 2.5×10^{-3} to 9.1×10^{-3} M. The $[\beta\text{-CD}]/[\text{FFN}]$ molar ratios were calculated by direct integration of appropriate signals. All the spectra consist of one set of concentration dependent resonances for each proton or group of equivalent protons, indicating a fast reversible exchange between free and complexed drug on the NMR time scale.

An examination of the β -CD proton signals in the ^1H NMR spectra of mixtures of FFN and β -CD revealed significant upfield shift changes in the H-3' and H-5' proton resonances, which are positioned inside the β -CD cavity, compared to pure β -CD. This clearly indicates the formation of FFN/ β -CD complex in solution, in analogy to previous reports. Figure 1 shows the expansions of the ^1H NMR spectral regions containing β -CD proton signals, in the absence as well as in the presence of FFN.

To have an insight into the detailed structure of the complex, the investigation of the chemical shift changes in the signals for the guest protons in the presence of β -CD is necessary and thus the unambiguous resonance assignment of the guest protons is required. The assignment of resonances of guest protons was made with help of COSY and ROESY spectral data. The signal appearing in the lowest field as a doublet at 7.482 was assigned to H-3 as it showed correlation peaks with H-6. The cross peaks between H-2 and H-11 and between H-1 and CH₃ support the resonance assignment for the *p*-substituted aromatic ring protons. The H-1 appeared relatively downfield compared to H-2 signal.

In the presence of β -CD, significant shift changes were observed in the signals for protons of all the three aromatic



Fexofenadine (FFN)

Table 1. ^1H NMR chemical shift change data of studied protons of FFN, in the presence of β -CD

$[\beta\text{-CD}]/[\text{FFN}]$	H-1	H-2, 4	H-3	H-5	CH ₃ -13
2.46	-0.060	-0.023	0.109	-0.003	0.068
1.76	-0.058	-0.028	0.089	-0.004	0.038
1.27	-0.042	-0.014	0.060	0.001	0.053
1.15	-0.040	-0.011	0.052	0.000	0.052
1.12	-0.044	-0.017	0.047	-0.005	0.039
0.68	-0.030	-0.010	0.026	-0.004	0.030

Negative values indicate upfield shift changes

rings of FFN (Table 1) confirming the penetration of all the aromatic rings into the β -CD cavity driven by hydrophobic interactions. The signals for H-1 and H-2 of *p*-substituted ring and H-4 of ring B exhibited upfield shifts while H-3 of aromatic ring B showed downfield shift. Moreover, the H-3 signal showed splitting in the presence of β -CD suggesting that both the B rings are not identical which is because the guest has a chiral center. The magnitude of the $\Delta\delta_{\text{H-3}}$ was greater than $\Delta\delta_{\text{H-1}}$ which means that the entry of the phenyl ring into the β -CD cavity is favored compared to *p*-substituted ring. The involvement of all the aromatic rings in

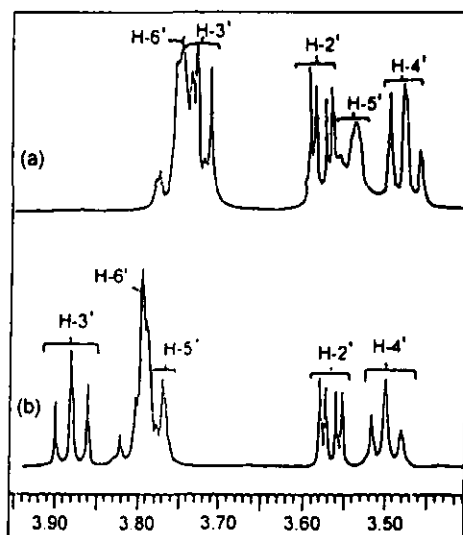


Figure 1. Part of the 500 MHz ^1H NMR spectra showing β -CD protons of the (a) 1 : 1.15 FFN/ β -CD mixture in comparison to (b) pure β -CD.

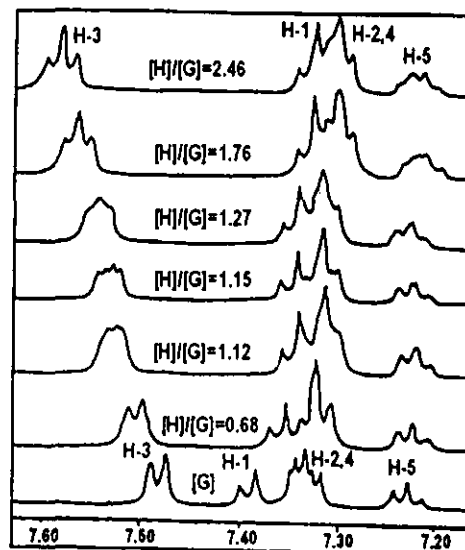


Figure 2. A part of ^1H NMR spectrum (500 MHz) showing aromatic protons of fexofenadine hydrochloride (FFN) in the absence as well as in the presence of varying amounts of β -CD.

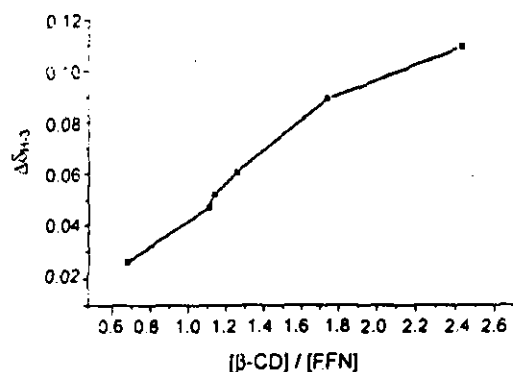


Figure 3. Mole ratio diagram of the chemical shift changes of FFN proton during titration with β -CD in D_2O . The concentration of the FFN was kept constant at 3.7×10^{-3} M.

complexation points to the existence of multiple equilibria in solution.^{19,20} Part of the spectra showing aromatic protons of the FFN, in the absence as well as in the presence of β -CD, are shown in Figure 2.

The stoichiometry of the complex was determined by mole ratio method, though Job's plot is considered better, because of poor solubility of FFN in D_2O . Figure 3 illustrates plot of $\Delta\delta$ vs mole ratio ($[\beta\text{-CD}]/[\text{FFN}]$) for H-3 of rings B. The $\Delta\delta$ values increase in a linear fashion till $[\beta\text{-CD}]/[\text{FFN}]=1$ suggesting 1 : 1 stoichiometry but since $\Delta\delta$ does not become constant and still keeps increasing even

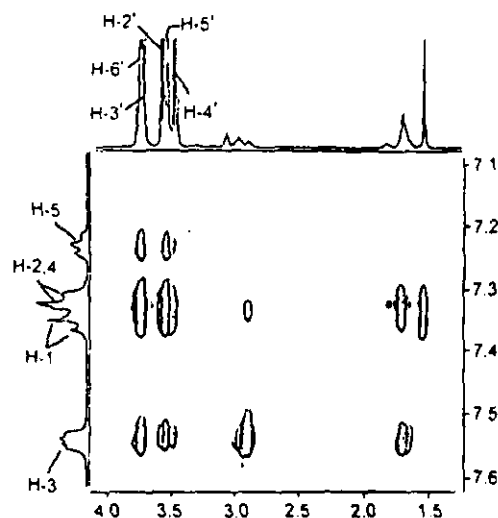


Figure 4. Expansion of the 500 MHz ROESY spectrum recorded with $T_m=300$ ms showing cross peaks of methyl and all the three aromatic ring protons with β -CD protons.

when $[\beta\text{-CD}]/[\text{FFN}]>1$ implies that the stoichiometry is not simply 1 : 1 but a combination of 1 : 1 and 1 : 2 (guest/host) complexes. The formation of 1 : 2 complex is favored when $[\beta\text{-CD}]/[\text{FFN}]>1$.

ROESY spectrum was recorded for a mixture of FFN and β -CD having molar ratio equal to 1.15 in D_2O to ascertain

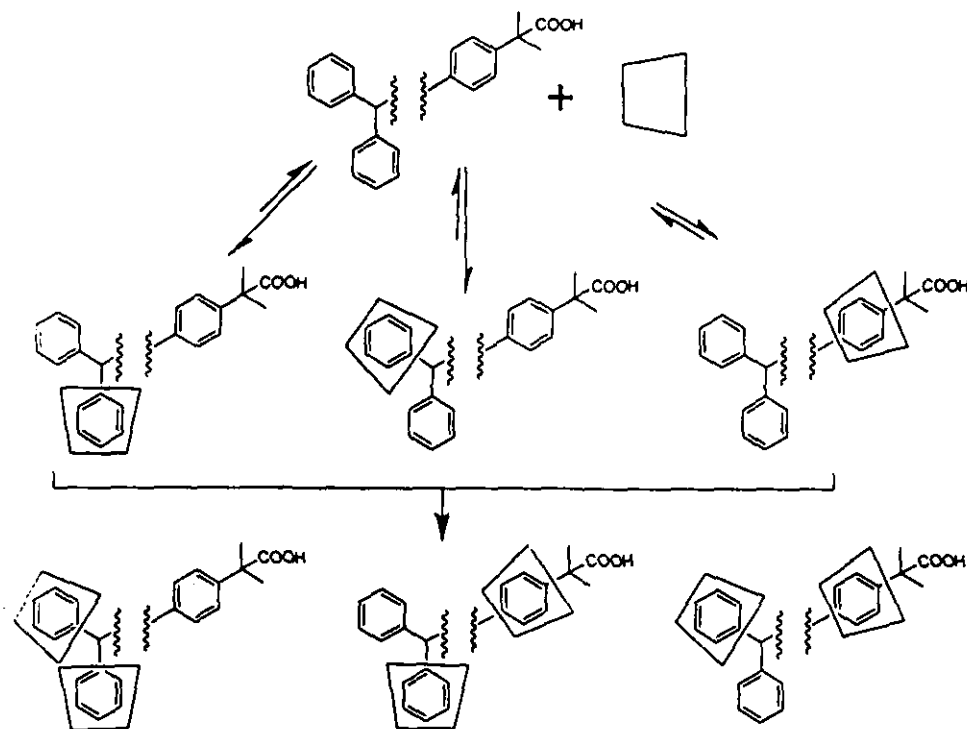


Figure 5. Schematic representation of all the possible inclusion complexes formed in D_2O between FFN and β -CD.

the existence of multiple equilibria in solution and to have an insight into the mode of penetration and orientation of the aromatic ring into the β -CD cavity. As shown in the Figure 4, the ROESY spectrum displayed correlation peaks between protons of all the aromatic ring protons with H-3' and H-5' of β -CD situated inside the cavity thus confirming beyond doubt the involvement of all the aromatic rings in the complexation. The cross peaks between H-3 of FFN and H-2',4' of β -CD, situated near wider rim clearly indicate that phenyl ring penetrates the cavity from wider rim side while *p*-substituted ring penetrates from narrower rim side since H-1 is showing cross peak with H-2',4' of β -CD. Figure 5 shows the proposed structures for all the possible FFN/ β -CD inclusion complexes present in solution.

Conclusion

^1H NMR titration studies of fexofenadine hydrochloride (FFN) with β -cyclodextrin (β -CD) in D_2O confirmed the presence of several inclusion complexes involving all the three aromatic rings. The structures for all the possible 1 : 1 and 1 : 2 complexes have been proposed taking into account the stoichiometry and ROESY spectral data. The mode of penetration has been clearly established.

Acknowledgements. Fexofenadine hydrochloride and cyclodextrin were very kindly provided by Surya Pharmaceutical Ltd, Chandigarh, India, and Geertui Haest, Cerestar Cargill, Belgium, respectively. Authors are highly grateful to Du Li, Department of Chemistry, Brigham Young University, Utah, USA and A. C. Kunwar, IIC, Hyderabad, India for their help in obtaining NMR data.

References

1. Simpson, K.; Jarvis, B. *Drugs* 2000, 59, 301.
2. Bender, M. L.; Komiyama, M. *Cyclodextrin Chemistry*; Springer Verlag: New York, 1978; pp 1.
3. Saenger, W. *Angew. Chem. Int. Ed. Engl.* 1980, 19, 344.
4. Szejtli, J. *Cyclodextrin Technology*; Kluwer: Dordrecht, 1988; pp 450.
5. Vogtle, F. *Supramolecular Chemistry: An Introduction*; John Wiley & Sons Ltd: New York, 1991.
6. Wenz, G. *Angew. Chem. Int. Ed. Engl.* 1994, 33, 803.
7. Esclusa-Diaz, M. T.; Gayo-Otero, M.; Perez-Marcos, M. B.; Villalato, J. L. *Int. J. Pharm.* 1996, 142, 183.
8. Obaidat, A. A.; Matalgah, S. M.; Najib, N. M. *Acta Pharm.* 2002, 52, 9.
9. Torres-Labandeira, J. J.; Blanco-Mendez, J.; Villa-Jato, J. L. *STP Pharm. Sci.* 1994, 4, 235.
10. Szejtli, J. *Med. Res. Rev.* 1994, 14, 353.
11. Schneider, H.-J.; Hacket, F.; Rudiger, V.; Ikeda, H. *Chem. Rev.* 1998, 98, 1755 and references cited therein.
12. Rekharsky, M. V.; Goldberg, R. N.; Schwarz, F. P.; Tewari, Y. B.; Ross, P. D.; Yamashoji, Y.; Inoue, Y. *J. Am. Chem. Soc.* 1995, 117, 8830.
13. Nakajima, T.; Sunagawa, M.; Hirohashi, T.; Fujioka, F. *Chem. Pharm. Bull. Jpn.* 1984, 32, 400.
14. Qi, Z. H.; Mak, V.; Diaz, L.; Grant, D. M.; Chang, C. J. *Org. Chem.* 1991, 56, 1537.
15. Neuhaus, D.; Williamson, M. In *The Nuclear Overhauser Effect in Structural and Conformational Analysis*; VCH Publishers: New York, 1989; p 1.
16. Szejtli, J. *Chem. Rev.* 1998, 98, 1743.
17. Ali, S. M.; Maheshwari, A.; Asmat, F. *Pharmazie* 2004, 59, 653.
18. Ali, S. M.; Asmat, F.; Maheshwari, A. *IL Farmaco* 2004, 59, 835.
19. Fronza, G.; Mele, A.; Redenti, E.; Ventura, P. *J. Pharm. Sci.* 1992, 81, 1162.
20. Yoshida, N. *J. Chem. Soc. Perk. Trans. 2* 1995, 2249.

COMPLEXATION OF ENALAPRIL MALEATE WITH β -CYCLODEXTRIN: NMR SPECTROSCOPIC STUDY IN SOLUTION

Syed Mashhood Ali*, Arti Maheshwari and Fahmeena Asmat

Department of Chemistry, Aligarh Muslim University, Aligarh 202002 (UP), India

Mamoru Koketsu

Division of Instrumental Analysis, Life Science Research Center, Gifu University, Gifu, 501-1193, Japan

Recebido em 28/3/05; aceito em 14/10/05; publicado na web em

A detailed NMR (^1H , COSY, ROESY) spectroscopic study of complexation of enalapril maleate with β -cyclodextrin was carried out. The ^1H NMR spectrum of enalapril maleate confirmed the existence of cis-trans equilibrium in solution, possibly due to hindered rotation along the amide bond. The cis-trans ratio remained almost the same in the presence of β -cyclodextrin but in one case it was found significantly different which suggests a catalytic role of β -cyclodextrin in the isomerization. ^1H NMR titration studies confirmed the formation of an enalapril- β -cyclodextrin inclusion complex as evidenced by chemical shift variations in the proton resonances of both the host and the guest. The stoichiometry of the complex was determined to be 2:1 (guest: host). The mode of penetration of the guest into the β -cyclodextrin cavity as well as the structure of the complex were established using ROESY spectroscopy.

Keywords: enalapril maleate; β -cyclodextrin; NMR spectroscopy.

INTRODUCTION

Enalapril maleate and its analogs are highly effective antihypertensive agents which act as angiotensin converting enzyme (ACE) inhibitors. ACE inhibitors prevent conversion of angiotensin I to II and act to widen the blood vessels resulting in easier pumping of blood by the heart. They are, therefore, used for the treatment of hypertension, congestive heart failure and to alleviate strain on heart damaged by heart attack^{1,2}. The solubility of enalapril maleate in water is poor but it is highly soluble in methanol.

The study of inclusion complex of pharmaceuticals with cyclodextrins (CDs) is a subject of great interest. Such studies involve structure determination of the complex besides the energetics of the complex formation. CDs are doughnut shaped molecules composed of 6, 7 or 8 α -D-glucose units, linked through α -1,4 bonds, called α , β and γ -CD, respectively. The primary hydroxyl groups are located at the narrow rim while wider rim is lined with secondary hydroxyl groups. The exterior of the CD_s is fairly hydrophilic due to the presence of hydroxyl groups while CD cavity is relatively hydrophobic. By virtue of their shape and hydrophobic nature of cavity, CD_s accommodate a variety of hydrophobic molecules, or part of it, inside their cavity through non-covalent interactions to form inclusion complexes³.

Inclusion of the guest into the CD cavity results in altered physico-chemical properties of the guest like water solubility, bioavailability, stability and volatility etc⁴. The majority of drug molecules are poorly water soluble and their biological absorption is, consequently, slow and far from being complete. Most drug molecules are ideal guests for CD_s because their polarity, size and structure enable them to penetrate into the cavity. Moreover, the inclusion complexes of pharmaceutical compounds with CD_s have several desirable properties. This is the reason why major portion of CD related studies is dedicated to pharmaceutical

applications. A variety of techniques are used, for characterization of inclusion complexes in terms of geometry and configurational preferences, of which solid state and high resolution NMR spectroscopy are most important⁵.

The formation of inclusion complex results in the shift changes in resonances of both the CDs and the guest protons⁶. The magnitude of $\Delta\delta$ is a critical function of position of proton in the molecule, size of CD cavity as well as host/guest ratio. In general, the inclusion of an aromatic ligand into the CD cavity causes major upfield shifts in the H-3' and H-5' of CD, situated inside the cavity, which is attributed to the ring current effect of the aromatic ring. The protons located outside cavity show negligible or trivial changes. Contrary to the upfield shift changes experienced by the CD protons, situated inside the cavity as a result of complexation, all the guest protons, and not only those included inside the CD cavity, generally show downfield shift changes upon complexation. This has been attributed, besides the shielding effect of the cavity, to other factors like changes in the microenvironment or conformational changes⁷. The preferred mode of penetration of guest into the cavity is from wider rim side but complexes in which the entry of the guest takes place from narrower rim side are also known⁸.

Simple ^1H NMR titration studies between CD_s and the guest confirmed the formation of inclusion complex. Moreover, valuable information regarding mode of penetration of guest into CD cavity, i.e. from narrower or wider rim side, stoichiometry and stability of the complex can be obtained by the treatment of ^1H NMR titration data⁹. Advanced 2D NMR techniques like NOESY and ROESY prove very helpful in unambiguous assignment of structure of the complex.

In continuation of our work on the NMR studies of inclusion complexes of pharmaceutical compounds with β -CD¹⁰, we report herein our results on the study of complexation of enalapril maleate with β -CD in solution by NMR spectroscopy.

EXPERIMENTAL PART

Enalapril maleate was obtained from Nebula Health Care Ltd, India, while β -CD was obtained from Cerestar, Belgium, and these

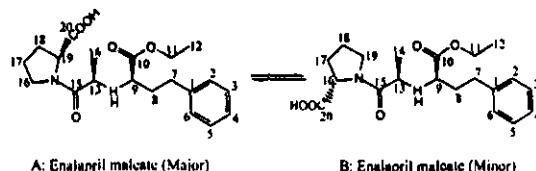
*e-mail: smashhoodali@yahoo.com

were used without further purification. ^1H NMR spectra were recorded on a JEOL 400 MHz instrument in D_2O at 25°C . The ^1H NMR measurements were carried out with 4 s relaxation delay, 0.73275969 Hz resolution and 16384 data points. 2D ROESY measurements were made with the experimental conditions as follows: scans 32, acquisition time 0.1470 s, pulse delay 2.3530 s and data points 512. The concentration of β -CD was kept constant at 10 mM while the concentration of enalapril maleate was varied from 11.4 to 52.6 mM. The H/G molar ratios were calculated by direct integration of appropriate signals. The chemical shift values, reported in δ (ppm), were calculated with reference to HDO peak at 4.7846.

RESULTS AND DISCUSSION

Two diastereomeric forms, (SSS)- and (RSS)-, of enalapril maleate, chemically known as 1-[(S)-1-ethoxycarbonyl-3-phenylpropyl]-L-alanyl-L-proline maleate, are known of which only (SSS)-diastereomer is used as drug¹¹. ^1H NMR spectra of enalapril maleate (SSS)-diastereomer, in the absence as well as in the presence of β -CD, showed that two geometrical isomers are in equilibrium in solution. Cis-trans isomerism is possible in the case of enalapril maleate due to hindered rotation along amide bond¹². The spectral assignment for the major (A) and minor (B) isomers was made with the help of COSY spectrum. The chemical shift data of the studied protons for pure enalapril maleate and enalapril maleate- β -CD mixtures is given in Table 1. The signals for the protons of three methylenic groups, for two isomers, at C-8, 17, 18 were found resonating as multiplets centered at 2.2700 ($3\text{H}_\text{A}+4\text{H}_\text{B}$), 1.9600 ($3\text{H}_\text{A}+1\text{H}_\text{B}$) and 1.7200 (1H_A). The signal for $\text{H}_\text{A}-19$ was observed resonating at 3.5328

in the case of pure enalapril maleate. In the presence of β -CD, this signal merged with β -CD protons and, therefore, its chemical shift changes could not be determined. The structures for the two isomers could be established by comparative study of the H-13, 14 and 16 signals. The major isomer was assigned structure (A) since H-13 and 14, being in close proximity of oxygen of $-\text{COOH}$ group should appear downfield compared to signals for the same protons of the minor isomer (B) which is in good agreement with earlier observation¹³. As expected, the signal for H-16 for major isomer (A) appears relatively upfield compared to that of minor isomer.



As expected, changes in the shapes as well as chemical shifts of enalapril maleate protons were observed in the presence of β -CD. All the protons, except H-11, exhibited downfield shift changes. The highfield shift in the H-11 signal may be due to hydrogen bonding between the hydrogen of the rim hydroxyl and $-\text{COOC}_2\text{H}_5$ group. The signal for H-11, which appeared as a quartet at 4.2321 in the spectrum of pure enalapril maleate, became complex in the presence of β -CD making it difficult to calculate chemical shift changes. The $\Delta\delta$ values for all the studied protons of the enalapril maleate are given in Table 1.

Table 1. ^1H NMR (400 MHz) Chemical shift (δ , ppm) data for Enalapril maleate (G) and chemical shift change ($\Delta\delta$) values for the studied protons of Enalapril maleate in the presence of β -cyclodextrin (H) in D_2O

Proton	[G]	[H]/[G]=0.875	[H]/[G]=0.750	[H]/[G]=0.560	[H]/[G]=0.335	[H]/[G]=0.190
$\text{H}_\text{A}-12$	1.2490	0.0092	0.0110	0.0110	0.0128	0.0092
$\text{H}_\text{B}-12$	1.2490	0.0183	0.0202	0.0202	0.0202	0.0147
$\text{H}_\text{A}-14$	1.4910	0.0302	0.0284	0.0247	0.0229	0.0174
$\text{H}_\text{B}-14$	1.5368	0.0275	0.0257	0.0232	0.0211	0.0156
$\text{H}_\text{A}-7$	2.7600	0.0700	0.0620	0.0500	0.0450	0.0350
$\text{H}_\text{B}-19$	3.4000	0.0300	0.0200	0.0150	0.0100	0.0020
$\text{H}_\text{B}-9$	3.8700	0.0530	0.0476	0.0421	0.0312	0.0310
$\text{H}_\text{A}-9$	3.9100	0.0608	0.0517	0.0462	0.0388	0.0278
$\text{H}_\text{B}-13$	4.0380	#	0.0290	0.0250	0.0220	0.0170
$\text{H}_\text{A}-13$	4.2620	0.0360	0.0333	0.0296	0.0280	0.0220
$\text{H}_\text{A}-16$	4.3800	0.0436	0.0300	0.0280	0.0262	0.0230
$\text{H}_\text{B}-16$	4.4200	0.0130	0.0100	0.0080	0.0050	0.0015
$\text{H}_\text{A,B}-2,4,6$	7.2550	0.0250	0.0200	0.0150	0.0120	0.0080
$\text{H}_\text{A,B}-3,5$	7.3360	0.0140	0.0140	0.0100	0.0090	0.0040

#could not be determined.

Table 2. ^1H NMR (400 MHz) chemical shift change ($\Delta\delta$) values for β -cyclodextrin protons in the presence of Enalapril maleate in D_2O

A/B ^a	[H]/[G]	H-1'	H-2'	H-3'	H-4'	H-5'	H-6'
73:27	0.875	-0.0055	0.0100	-0.0402	-0.0019	-0.0730	-0.0073
60:40	0.750	-0.0073	0.0064	-0.0403	-0.0037	-0.0738	-0.0091
61:39	0.560	-0.0091	0.0046	-0.0439	-0.0055	-0.0798	-0.0128
59:41	0.335	-0.0128	0.0046	-0.0567	-0.0092	-0.0998	-0.0183
64:36	0.190	-0.0201	0.0037	-0.0751	-0.0147	-0.1428	-0.0311

^acis / trans ratio of enalapril maleate. Negative values indicate high field shift.

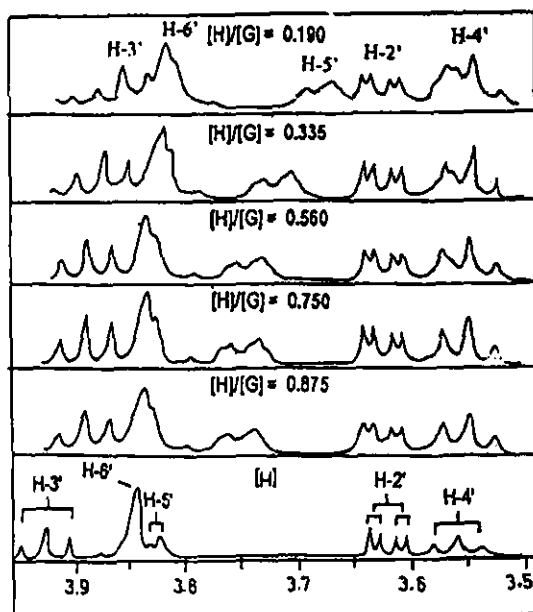


Figure 1. Partial ^1H NMR (400 MHz) spectra showing signals for the protons of β -CD [H] in the absence as well as in the presence of varying amounts of enalapril maleate [G]. Note the large upfield shifts of H-3' and H-5' in sharp contrast to the small shift changes observed for H-2', H-4' and H-6'.

^1H NMR spectra of β -CD in the presence of enalapril maleate displayed significant highfield shift changes in the signal for H-3' and H-5' while the peaks for other protons showed negligible $\Delta\delta$. The $\Delta\delta$ data for all the β -CD protons compared to pure β -CD has been given in Table 2. The progressive upfield shift of H-3' and H-5' resonances in the presence of varying amounts of enalapril maleate are shown in Figure 1 which shows the part of the ^1H NMR spectra containing β -CD signals.

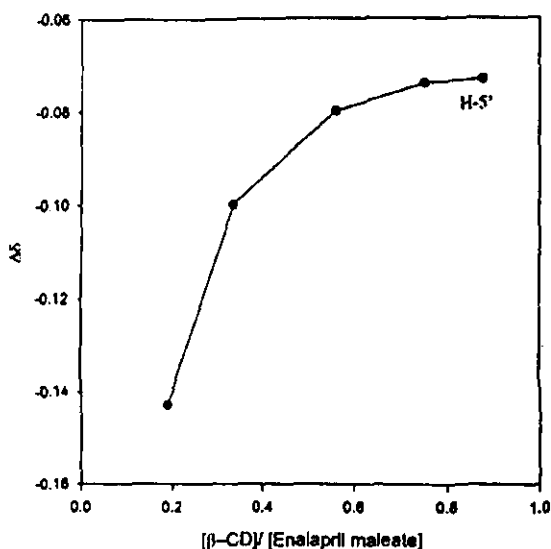


Figure 2. Mole ratio plot of chemical shift change for H-5' ($\Delta\delta_{\text{H-5'}}$) of β -CD during titration with enalapril maleate

The upfield shifts observed for the signals of H-3' and H-5', situated inside the β -CD cavity, and concomitant downfield shift changes for enalapril maleate protons in the ^1H NMR spectra of mixtures of enalapril maleate and β -CD are a clear indication of complexation between enalapril maleate and β -CD, in analogy to previous studies^{3,6}. Furthermore, the magnitude of shift changes of these β -CD protons increased with the increase in the concentration of enalapril maleate. The stoichiometry of the complex was determined by mole ratio method¹⁴. The plot $\Delta\delta_{\text{H-5'}}$ vs H/G (Figure 2) shows flattening of the slope of the curve at a molar ratio of around 0.5 suggesting a 1:2 stoichiometry for the β -CD-*enalapril maleate* complex.

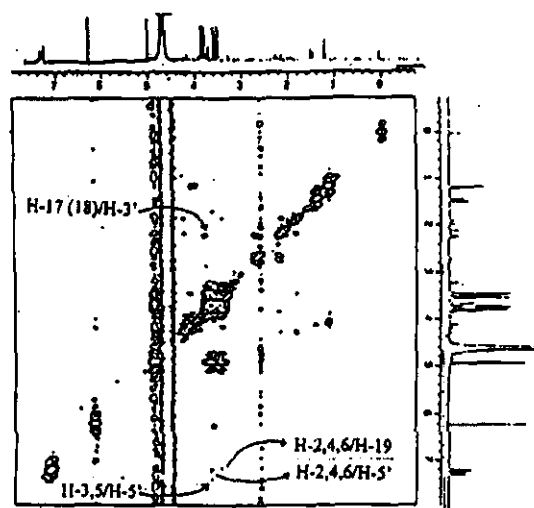


Figure 3. 2D ROESY spectrum (400 MHz) of mixture of β -CD [H] and enalapril maleate [G] ([H]/[G]=0.807). The marked cross peaks support structure for 1:2 β -CD-*enalapril maleate* complex

ROESY¹⁵ spectrum (Figure 3) was recorded for a mixture of enalapril maleate and β -CD ([H]/[G]=0.807) to study the mode of penetration of two guest molecules into a β -CD cavity. Intermolecular cross peaks observed between the aromatic protons of the guest and H-5' of β -CD and no cross peaks observed between the aromatic protons and H-3' confirm the shallow penetration of the aromatic ring into the β -CD cavity from narrower rim side¹¹. Moreover, Cross peak between H-19 and H-2, 4, 6 of enalapril maleate confirmed that aromatic ring of one guest molecule and 5-membered ring of another molecule share the same β -CD cavity which is in agreement with the 1:2 host:guest stoichiometry of the complex. Cross peak between H-17 (18) and H-3' was also observed which confirms that 5-membered ring enters the β -CD cavity from wider rim side. Figure 4 shows an expanded region of the ROESY spectrum containing cross peaks between the β -CD and enalapril maleate protons. The proposed structure for the 1:2 β -CD-*enalapril maleate* complex is shown in Figure 5.

It is important to mention here that though the *cis-trans* ratio for enalapril maleate, as determined from the integration of 14-CH₂ doublet, remained almost unchanged, in the presence of β -CD, in four samples. However, there was a significant change observed in one of the sample (Table 2). This can only be explained in terms of catalysis by the β -CD. However, this needs further investigation.

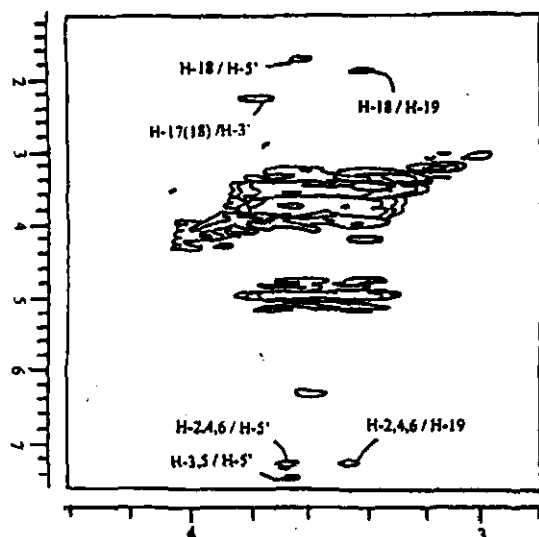


Figure 4. Expansion of part 2D ROESY spectrum showing crosspeaks between enalapril maleate and β -CD

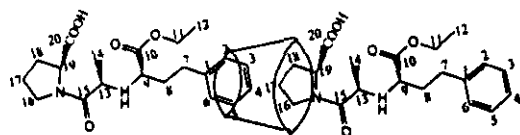


Figure 5. Proposed structure for 1:2 β -cyclodextrin-enalapril maleate complex

CONCLUSION

Enalapril maleate, which exists in two geometrical forms in solution, forms a 1:2 host-guest inclusion complex with β -CD in the concentration range studied. The aromatic ring of one guest

molecule enters the β -CD cavity from narrower rim side while 5-membered ring penetrates through wider rim side as evidenced by ROESY spectrum. The structure for the complex has been proposed.

ACKNOWLEDGEMENT

The authors are thankful to Nebulae Health Care Ltd., India, for providing the gift sample of pure enalapril maleate and thanks are also extended to Mr. G. Haest, Cerestar Application Center and Pharma Specialties, Belgium, for providing β -cyclodextrin.

REFERENCES

1. Cleary, J. D.; Taylor, J. W.; *Drug Intell. Clin. Pharm.* 1986, 20, 177.
2. Todd, P. A.; Heel, R. C.; *Drugs* 1986, 31, 198.
3. Vogtle, F.; *Supramolecular Chemistry: An Introduction*, John Wiley and Sons Ltd.: New York, 1991, p. 135.
4. Szajli, J.; *Medicinal Research Reviews* 1994, 14, 364.
5. Schneider, H.-J.; Hackett, F.; Rudiger, V.; Ikeda, H.; *Chem. Rev.* 1998, 98, 1755.
6. Demarco, P. V.; Thakkar, A. L.; *J. Chem. Soc. Chem. Commun.* 1970, 2.
7. Rekharsky, M. V.; Goldberg, R. N.; Schwarz, F. P.; Tewari, Y. B.; Ross, P. D.; Yamashoji, Y.; Inoue, Y.; *J. Am. Chem. Soc.* 1995, 117, 8830.
8. Nakajima, T.; Sunagawa, M.; Hirohashi, T.; Fujioka, K.; *Chem. Pharm. Bull.* 1984, 32, 383.
9. Ali, S. M.; Maheshwari, A.; Asmat, F.; *Die Pharmazie* 2004, 59, 653.
10. Ali, S. M.; Asmat, F.; Maheshwari, A.; *Il Farmaco* 2004, 59, 835.
11. Slavinska, V.; Sile, D.; Chipens, G. I.; Balodis, Y.; Rozenthal, G.; Venteris, K.; Lukevics, E.; *Chem. Heterocyc. Compd.* 2003, 39, 1579.
12. Fronza, G.; Mele, A.; Redente, E.; Ventura, P.; *J. Org. Chem.* 1996, 61, 909.
13. Wyvratt, M. J.; Tristram, E. W.; Ikeler, T. J.; Lohr, N. S.; Joshua, H.; Springer, J. P.; Arison, B. H.; Patchett, A. A.; *J. Org. Chem.* 1984, 49, 2816. ¹H NMR (D₂O) δ 1.30 (t, J=7 Hz, OCH₂CH₃), 1.54 (minor), 1.59 (major, d, J=6.5 Hz, Ala-CH₃), 1.75 (minor), 2.02 (major, m, Pro- γ -CH₂), 2.02 (m, Pro- β -H), 2.33 (m, Pro- β -H, PhCH₂CH₂), 2.82 (m, PhCH₂), 3.48 (minor), 3.60 (major, Pro- δ -CH₂), 3.96 (m, PhCH₂CH₂CH), 4.09 (minor), 4.33 (major, m, q, J = 6.5 Hz, Ala- α -H), 4.27 (q, J = 7 Hz, OCH₂CH₃), 4.44 (m, Pro- α -H), 6.37 (s, CH=CH), 7.39 (m, ArH_{2,4}). The rotameric distribution is indicated by *minor/major* terminology.
14. Yannakopoulou, K.; Ripseester, J. A.; Mavridis, I. M.; *J. Chem. Soc., Perkin Trans. 2* 2002, 1639.
15. Bednarek, E.; Bocian, W.; Poznanski, J.; Sitkowski, J.; Sadlej-Sosnowska, N.; Kozerski, L.; *J. Chem. Soc., Perkin Trans. 2* 2002, 999.



Article original

NMR spectroscopy of inclusion complex of D-(-)-chloramphenicol with β -cyclodextrin in aqueous solution

Syed Mashhood Ali *, Fahmeena Asmat, Arti Maheshwari

Department of Chemistry, Aligarh Muslim University, Aligarh-202002 UP, India

Received 9 March 2004; accepted 12 May 2004

Available online 19 August 2004

Abstract

¹HNMR spectroscopic study in D₂O of mixtures of D-(-)-chloramphenicol (guest), present in two tautomeric forms in solution, and β -cyclodextrin (host) revealed the formation of 1:1 inclusion complex in which aromatic ring of the guest is tightly held by the host cavity. There seems no discrimination between the aromatic rings of two tautomers by the host.
© 2004 Elsevier SAS. All rights reserved.

Keywords: ¹HNMR; Inclusion complexes; β -Cyclodextrin; D-(-)-Chloramphenicol

1. Introduction

Cyclodextrins (CDs) are among the most widely used hosts for their ability to bind organic molecules in aqueous solution and in the crystalline state by non-covalent interactions [1]. The internal diameter of their torus shaped cavity and size of the guest molecule are of primary significance for the formation of inclusion complexes. The ability of CDs to hold a variety of guests into their hydrophobic cavity has rendered them very useful models for studying topochemistry and catalytic mechanism of enzymes [2]. Inclusion compounds have different physico-chemical properties, such as altered solubility, reduced volatility, reduced/enhanced stability, modified chemical reactivity and altered bio-availability as compared to free molecule, and that is why CDs are widely used in pharmaceutical applications as a vehicle for drug delivery of poorly water-soluble drugs [3,4]. NMR spectroscopy is the most reliable technique [5], of all, for the study of inclusion phenomenon because both the host and guest molecules are observed simultaneously. NMR studies of CD-complexes in solution generally show rapid exchange of guest molecule between the hosts resulting in averaged shifts and no distinct species on the NMR time-scale. However, valuable information regarding the inclusion

of the guest into the CD-cavity, their stoichiometry and stability can be extracted through treatment of the data from simple ¹HNMR experiments through the variation of the chemical shifts for different protons [6–8]. Extensive studies on the inclusion complexes of pharmaceutical compounds with β -CD have been reported [3,4,9]. In continuation with our work on the complexation behaviour of pharmaceuticals with β -CD [10], we report here our results on the study of inclusion complex of D-(-)-chloramphenicol, an antibacterial drug having a wide spectrum of activity against gram positive and gram negative cocci and bacilli (including anaerobes) [11], with β -CD in solution by NMR spectroscopy.

2. Results

¹HNMR spectra were recorded on a 300 MHz instrument at 25 °C for five samples with [H]/[G] molar ratios ranging from 2.31 to 1.27 which were determined by direct integration of the NMR signals. The anomeric proton (H-1) of β -CD was used as internal reference throughout this work. The use of this proton signal as a reference is justified because the water signal is too wide; H-3, H-5 signals show largest chemical shift changes on complexation; H-2, H-4 signals may also be affected though situated outside the cavity. The location of anomeric proton ensures the smallest (if any) induced shift.

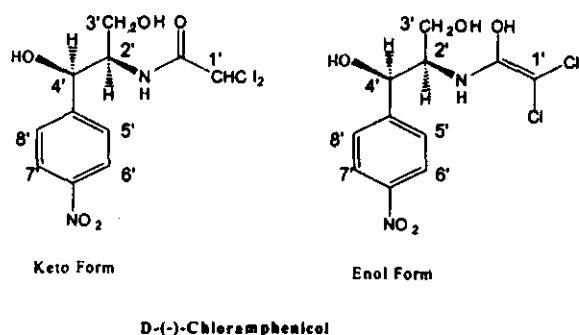
The signals for the protons of the drug and β -CD did not interfere with each other in any of the spectrum. ¹HNMR

* Corresponding author.

E-mail address: smashhoodali@yahoo.com (S. Mashhood Ali).

spectrum of each sample denoted largest chemical shift changes ($\Delta\delta$) for H-3 and H-5 protons of the β -CD while the other protons of the β -CD showed negligible deviations in the presence of guest. The chemical shift change ($\Delta\delta$) data for various protons of the β -CD in the presence of varying amounts of drug is given in Table 1. Part of the NMR spectra of samples A–E containing signals for the protons of β -CD is shown in Fig. 1. The progressive upfield shift in the signals for H-3 and H-5 protons of β -CD with the increase in the concentration of guest, is quite prominent.

A cursory examination of the signals for drug protons, in the absence as well as in the presence of β -CD, clearly indicates that two tautomeric forms, namely keto and enol, are present in solution in the approximate ratio of 3:2, as determined by the integration of NMR signals appearing separately for the two tautomers.



The presence of two tautomers of drug molecule was evident from the signal for H-1' proton which integrated for two-third of a proton. Moreover, the signals for H-3' and H-4' protons appeared separately for the two tautomers while aromatic protons were observed together as a pair of doublets having AB pattern. In the presence of β -CD, signal for H-5',8' aromatic protons of drug moved progressively downfield with the increase in the concentration of β -CD while an upfield shift in the H-6',7' aromatic protons signal was observed, though small in magnitude. The multiplet for H-2' proton also exhibited large downfield shift in the presence of β -CD while H-3' signal did not show any appreciable deviation. The signal for H-4' of keto form also moved downfield but no significant change for the same proton of enol form was observed. Table 2 shows the chemical shift change ($\Delta\delta$) data for the D-(-)-chloramphenicol protons and part of the NMR spectra showing the chemical shift changes for various protons of the drug in the presence as well as absence of β -CD is given in Fig. 2.

Table 1

¹HNMR Chemical shift changes ($\Delta\delta$) of β -cyclodextrin protons in the presence of D-(-)-chloramphenicol

Sample	[H]/[G]	H-2	H-3	H-4	H-5	H-6	H-5/H-3
A	2.31	0.002	-0.041	0.004	-0.051	0.000	1.24
B	1.95	-0.002	-0.045	-0.003	-0.054	-0.001	1.20
C	1.56	0.003	-0.054	0.001	-0.069	-0.002	1.27
D	1.38	-0.003	-0.061	-0.002	-0.081	-0.002	1.33
E	1.27	0.004	-0.071	-0.001	-0.098	0.006	1.40

Negative values indicate high field shift.

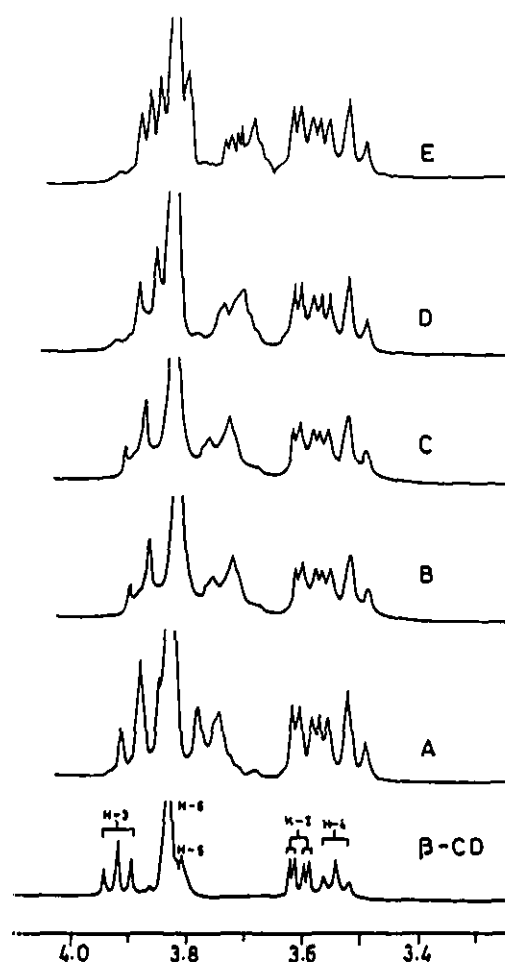


Fig. 1. Part of ¹HNMR spectra (300 MHz) containing signals for β -CD proton in the presence of D-(-)-chloramphenicol (A–E) and ¹HNMR (500 MHz) spectrum of β -CD.

3. Discussion

β -Cyclodextrin is a truncated right cylindrical cone shaped molecule, 7.9×10^{-8} cm high, with a hollow tapered cavity whose top and bottom dimensions are 6.5×10^{-8} and 6.0×10^{-8} cm [12]. The most likely mode of complexation of the guest involves insertion of the less polar portion into the cavity of the β -CD from the wider side resulting in major upfield shifts in the signals for H-3 and H-5 protons of β -CD, since these are positioned inside the cavity. This is attributed

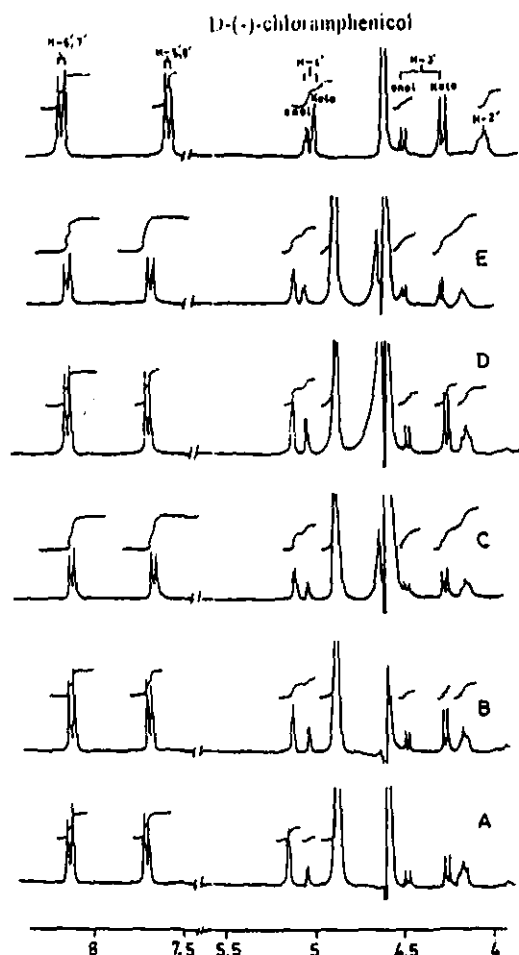


Fig. 2. Part of ^1H NMR spectra (300 MHz) containing signals for D-(-)-chloramphenicol protons in the presence (A-E) and absence of β -CD.

to the ring current of the aromatic ring of the guest molecule that is included into the cavity [13,14]. The other β -CD protons located outside the cavity show either no or negligible changes in their chemical shifts upon complexation. On the other hand, signals for most of the guest protons show downfield shift changes upon complexation with β -CD but the magnitude of the shifts is much smaller compared to β -CD protons. In a detailed thermodynamic and NMR spectroscopic study of the interaction of CDs with a variety of model substrates, Rekharsky et al. [15] demonstrated that the

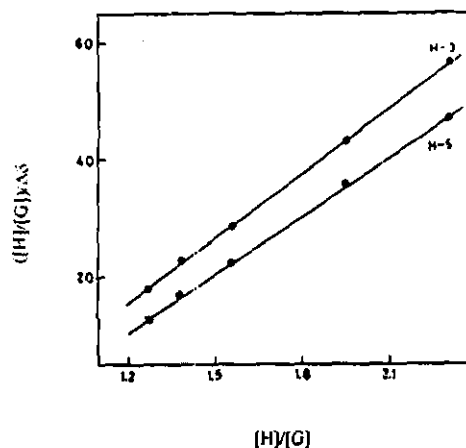


Fig. 3.

magnitude of the upfield shifts in the signals for H-5 and H-3 protons of CDs, $\Delta\delta(\text{H-5})$ and $\Delta\delta(\text{H-3})$, and their relative ratios, $\Delta\delta(\text{H-5})/\Delta\delta(\text{H-3})$, can be used as a quantitative measure of the stability of the complex and the depth of inclusion of the guest into the cavity, respectively.

It can be argued, in analogy to earlier reports [15–17], that the observed highfield shifts in the signals for H-5 (dominant) and H-3 (subordinate) protons are clearly indicative of the penetration of the aromatic ring into the β -CD cavity from the wider side, and the formation of a tight-fitting complex between the guest and β -CD as evidenced by high $\Delta\delta(\text{H-5})$ and $\Delta\delta(\text{H-3})$ values. Also, the values (1.20–1.40) for $\Delta\delta(\text{H-5})/\Delta\delta(\text{H-3})$ ratios (Table 1) are suggestive of deep insertion of the aromatic ring of the guest into the host cavity [15]. The stoichiometry of the complex was determined by plotting $\Delta\delta(\text{H-5})$ and $\Delta\delta(\text{H-3})$ vs. $[\text{H}]/[\text{G}]$ which gave slightly curved lines. However, a modified Benesi and Hildebrand [18] plot of chemical shift changes for H-3 and H-5 protons in the form of $([\text{H}]/[\text{G}])/\Delta\delta(\text{H-3 or 5})$ vs. $[\text{H}]/[\text{G}]$ gave excellent linear fits (Fig. 3) supporting 1:1 complexation. There seems no discrimination by the host between the two tautomeric forms of the guest as the signals for aromatic protons of both the tautomers appear together as a pair of doublets in the presence as well as in the absence of host. The chemical shift changes that take place in the signals for aromatic protons, upon complexation with β -CD, also give valuable information regarding the depth of penetration beside the values for $\Delta\delta(\text{H-5})/\Delta\delta(\text{H-3})$ ratios. All the aromatic

Table 2

^1H NMR chemical shift changes ($\Delta\delta$) for various protons of D-(-)-chloramphenicol in the presence of β -CD

Sample	[H]/[G]	H-2'	H-3'		H-4'		H-5',8'	H-6',7'
			Keto	Enol	Keto	Enol		
A	2.31	0.130	0.009	0.023	0.161	0.032	0.093	-0.028
B	1.95	0.128	0.006	0.022	0.154	0.029	0.086	-0.027
C	1.56	0.115	0.005	0.022	0.134	0.026	0.082	-0.025
D	1.38	0.108	0.004	0.021	0.123	0.024	0.079	-0.022
E	1.27	0.086	0.003	0.020	0.105	0.020	0.078	-0.021

Negative values indicate high field shift.

protons of the phenyl ring show downfield shift upon complexation [16], but when a polar group, present at para-position of the aromatic ring, enters the hydrophobic cavity of β -CD it passes through the cavity and protrudes out of the smaller rim. In such cases, the aromatic protons ortho to polar group exhibit upfield shift while those in meta position move downfield [19]. The chemical shift changes in the aromatic protons of the guest, in our case, also point to the nitro group protruding out of the smaller rim and exposed to solvent. The difference in the chemical shift changes for H-4' proton of keto and enol forms of the guest molecule may be due to different environment but is not clearly understood.

Finally, the ^1H NMR spectroscopic study, in solution of D-(-)-chloramphenicol with varying amounts of β -CD revealed the formation of a 1:1 tight fitting inclusion complex formed by the inclusion of the aromatic ring into the hydrophobic cavity of the β -CD. There is no evidence of discrimination by the host between the two tautomeric forms of the guest.

Acknowledgements

SMA wishes to express deep appreciation to an old friend Dr. Desh Deepak, Department of Chemistry, Lucknow University, Lucknow for the help in obtaining NMR spectra.

References

- [1] J.W. Steed, J.L. Atwood, *Supramolecular Chemistry*, Wiley-VCH, Weinheim, 2000, pp. 1.
- [2] M.L. Bender, *Enzyme Mechanisms*, Royal Society of Chemistry, Letchworth, 1988, pp. 1.
- [3] K. Aithal, U.N. Shrinivas, Physicochemical study of ciprofloxacin with β -CD, *Pharm. Sci.* 2 (1996) 451–455.
- [4] K. Fromming, J. Szejtli, *Cyclodextrin in Pharmacy*, Kluwer Academic, Dordrecht, 1994, pp. 1.
- [5] H.J. Schneider, F. Hacket, V. Rudiger, NMR studies of cyclodextrins and cyclodextrin complexes, *Chem. Rev.* 98 (1998) 1755–1785.
- [6] I. Suzuki, K. Obata, J.I. Auzai, H. Ikeda, A. Ueno, Crown ether-tethered cyclodextrins: superiority of the secondary-hydroxy side modification in binding tryptophan, *J. Chem. Soc. Perkin-II* (2000) 1705–1710.
- [7] F.J.B. Veiga, C.M. Fernandes, R.A. Carvalho, Molecular modeling and ^1H NMR: ultimate tools for the investigation of tolbutamide: β -CD and tolbutamide: hydroxypropyl- β -CD complexes, *Chem. Pharm. Bull.* 49 (2001) 1251–1286.
- [8] R. Wimmer, F.L. Aachmann, K.L. Larsen, S.B. Petersen, NMR diffusion as a novel tool for measuring the association constant between cyclodextrin, *Carbohydr. Res.* 337 (2002) 841–849.
- [9] F.A. Menard, M.G. Dedhiya, C.T. Rhodes, Physicochemical aspects of the complexation of some drugs with cyclodextrins, *Drug Dev. Ind. Pharm.* 16 (1990) 91–113.
- [10] S.M. Ali, A. Maheshwari, F. Asmat, Complexation of roxatidine acetate hydrochloride with β -cyclodextrin: NMR spectroscopic study, *Die Pharmazie*, 8 (2004) (in press).
- [11] M.H. Brooks, R.L. Smith, D.L. Macalady, Inhibition of existing denitrification enzyme activity by chloramphenicol, *Appl. Environ. Microbiol.* 58 (1992) 1746–1753.
- [12] S. Li, W.C. Purdy, Cyclodextrin and their application in analytical chemistry, *Chem. Rev.* 92 (1992) 1457–1470.
- [13] M. Komiyama, H. Hirai, Time averaged conformation of the inclusion complexes of β -cyclodextrin with *tert*-butylphenols, *Chem. Lett.* 12 (1980) 1467–1470.
- [14] M. Komiyama, H. Hirai, Relationship between the cyclodextrin catalysis in the cleavages of phenyl acetates and the time averaged conformation of the inclusion complexes, *Chem. Lett.* 12 (1980) 1471–1474.
- [15] M.V. Rekharsky, R.N. Goldberg, F.P. Schwarz, Y.B. Tiwari, P.D. Ross, Y. Yamashoji, Y. Inoue, Thermodynamic and nuclear magnetic resonance study of the interactions of α - and β -cyclodextrin with model substances. Phenylethylamine, ephedrine and related substances, *J. Am. Chem. Soc.* 117 (1995) 8830–8840.
- [16] Z.H. Qi, V. Mak, L. Diaz, D.M. Grant, C.J. Chang, Molecular recognition: β -cyclodextrin and penicillin V inclusion complexation, *J. Org. Chem.* 56 (1991) 1537–1542.
- [17] R.J. Bergeron, R. Rowan, The molecular disposition of sodium *p*-nitrophenolate in the cavities of cycloheptaamylose and cyclohexaamylose in solution, *Bioorg. Chem.* 5 (1976) 423–436.
- [18] H.A. Benesi, J.H. Hildebrand, Interaction of I with aromatic hydrocarbon, *J. Am. Chem. Soc.* 71 (1949) 2703–2707.
- [19] K. Kano, R. Nishiyabu, T. Asada, Y. Kuroda, Static and dynamic behaviour of 2:1 inclusion complexes of cyclodextrins and charged porphyrins in aqueous organic media, *J. Am. Chem. Soc.* 124 (2002) 9937–9944.



Complexation of fluoxetine hydrochloride with β -cyclodextrin. A proton magnetic resonance study in aqueous solution

Syed Mashhood Ali ^{a,*}, Fahmeena Asmat ^a, Arti Maheshwari ^a, Mamoru Koketsu ^b

^a Department of Chemistry, Aligarh Muslim University, Aligarh 202002, UP, India

^b Division of Instrument Analysis, Life Science Research Center, Gifu University, Gifu 501-1193, Japan

Received 1 December 2004; received in revised form 17 February 2005; accepted 5 March 2005

Available online 25 April 2005

Abstract

A proton magnetic resonance spectroscopic study in D₂O of mixtures of fluoxetine hydrochloride (guest) with β -cyclodextrin (host) revealed the existence of two different equilibria for 1:1 inclusion complexes in which –CF₃ substituted ring of the guest is more tightly held by the host cavity. The structures of the two complexes have been proposed which are supported by 2DROESY spectral data. The dissociation constant was also determined.

© 2005 Elsevier SAS. All rights reserved.

Keywords: Inclusion complexes; β -Cyclodextrin; Fluoxetine hydrochloride; ¹H NMR; ROESY

1. Introduction

Cyclodextrins (CDs) are polymeric carbohydrates (cyclic oligomers) differing from one another in number of glucopyranose units in their structure and are referred as α -, β - and γ -CD having 6–8 units, respectively [1]. Due to formation of α -1,4-glycosidic linkage, CDs are doughnut shaped molecules, possessing hollow cavities which can accommodate a variety of guests in aqueous solution and in the crystalline state by non-covalent interactions [2]. The presence of hydroxyl groups on the exterior surface of CDs make them fairly polar while the interior of the cavity is relatively non-polar. The slightly hydrophobic character of the inner cavity provides a driving force for host–guest complexation with similarly apolar guest molecules. The study of inclusion complexes of pharmaceuticals with CDs have received considerable attention due to altered physical and chemical properties of the included guest molecules [3]. Several drugs are already approved and marketed in CD-complexed form and considering the strict requirements for approval of a new chemical entity, the study of inclusion complexes of drug molecules with CDs has become a subject of great interest [4].

In continuation of our work on the NMR spectroscopic studies of β -CD-complexes of pharmaceutical compounds

[5,6], we report here our results on the study of inclusion complex of racemic fluoxetine hydrochloride (G) with β -CD (H). Fluoxetine hydrochloride, commercially known as 'Prozac', is an antidepressant, antiobsessional and anti-bulimic drug. It is a potent and selective inhibitor of serotonin uptake. This uptake inhibition by fluoxetine hydrochloride enhances serotonergic function. As a consequence the serotonin receptors are desensitized or down regulated after long-term fluoxetine hydrochloride administration. It does not appear to cause down regulation of post synaptic beta-adrenergic receptors or a decrease in beta-adrenergic-stimulated cyclic adenosine monophosphate generation as do other antidepressant medications [7].

2. Results

All the NMR spectra were recorded on a 500 MHz Bruker instrument at 25 °C in D₂O. The H-1' signal of β -CD is used as a reference throughout this work [8] and chemical shift values are reported in ppm. ¹H NMR spectra for five mixtures of fluoxetine hydrochloride and β -CD, having [H]/[G] molar ratios ranging from 0.40–1.40, as determined by the integration of appropriate signals, were recorded. The amount of guest was kept constant (1×10^{-2} mmol) while amount of β -CD was varied. These samples were then dissolved in 0.5 ml D₂O. ROESY (500 MHz) experiment was performed on a

* Corresponding author.

E-mail address: smashhoodali@yahoo.com (S.M. Ali).

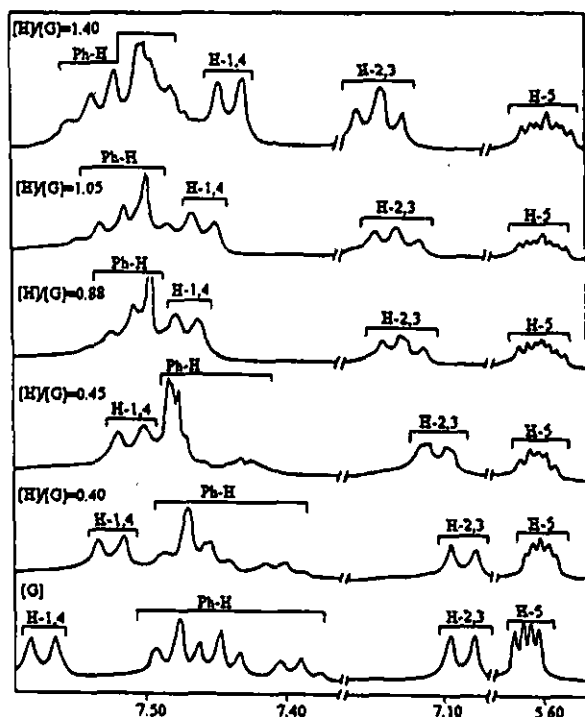


Fig. 1.

sample having [H]/[G] molar ratio as 0.45 with a mixing time of 225 ms at 25 °C. All the spectra contained one set of resonances suggesting a fast equilibrium between free and the complexed state, on the NMR time scale.

The signals for the protons of fluoxetine hydrochloride and β -CD did not interfere in any of the spectra. ^1H NMR spectra of fluoxetine hydrochloride, in the absence of β -CD, exhibited a pair of doublets, each integrating for two protons and showing A_2B_2 pattern, at 7.091 ($J = 8.95$ Hz) and 7.573 ($J = 8.75$ Hz) which were assignable to H-2,3 and H-1,4, respectively, of the trifluoromethyl substituted aromatic ring. The signal for H-8 of phenyl ring appeared as a triplet at 7.393

($J = 7.20$ Hz) while signals for remaining phenyl ring protons appeared merged in the region 7.430–7.505. A singlet at 2.779 for three protons was due to N-CH_3 . A doublet of doublet ($J_1 = J_2 = 4.25$ Hz) at 5.618 was ascribable to benzylic proton (H-5). A pair of multiplets centered at 2.364 and 2.463, each integrating for one proton, was assigned to methylenic protons (H-11) while signals for methylenic H-12 were found resonating as a pair of multiplets centered at 3.261 and 3.346.

Prominent changes in the nature and position of signals for most of the protons of fluoxetine hydrochloride were observed in the presence of β -CD which increased with the increase in the concentration of β -CD. The signals for the phenyl ring protons moved downfield and became complex making it difficult to calculate chemical shift change values in the presence of β -CD. The signals for H-2,3 of trifluoromethyl substituted aromatic ring exhibited downfield shifts with the increase in the concentration of β -CD and signals for two enantiomers started separating. A very significant upfield shift was observed in the H-1,4 proton signal in the presence of β -CD. The peaks for H-5 proton of the two enantiomers also separated in the presence of β -CD. Downfield shift changes were also observed in the signals for two methylenic groups of the drug in the presence of β -CD. Part of spectra containing studied signals of fluoxetine hydrochloride are shown in Fig. 1 while chemical shift change values for various protons of the guest are given in Table 1.

In all the spectra of mixtures of fluoxetine hydrochloride and β -CD, the signals for H-5' and H-3', situated inside the β -CD cavity, exhibited high field shifts compared to pure β -CD; the H-5' shifts being dominant while H-3' shifts as subordinate. The signal for the H-2' proton moved significantly downfield in the presence of fluoxetine hydrochloride. Though signals H-4' and H-6' also displayed downfield shifts, these were not very significant. The chemical shift change ($\Delta\delta$) values for β -CD protons are given in Table 2. The pattern of the spectral region containing β -CD signals, in the spectra of mixtures of fluoxetine hydrochloride and β -CD,

Table 1

^1H NMR (500 MHz) chemical shift change ($\Delta\delta$) values for various protons of fluoxetine hydrochloride in the presence of β -CD in D_2O at 25 °C

Sample	[H]/[G]	H-1,4	H-2,3	H-5	H-11a	H-11b	H-12a	H-12b	H-13
A	1.40	-0.138	0.047	-0.021	0.131	0.093	0.005	0.040	0.050
B	1.05	-0.115	0.040	-0.019	0.119	0.088	0.060	0.047	0.042
C	0.88	-0.102	0.038	-0.018	0.112	0.082	0.041	0.038	0.041
D	0.45	-0.060	0.016	-0.013	0.050	0.035	0.022	0.016	0.010
E	0.40	-0.056	-0.003	-0.016	0.025	0.013	0.104	0.005	0.010

Negative values indicate upfield shift.

Table 2

^1H NMR (500 MHz) chemical shift change ($\Delta\delta$) data for β -CD protons in the presence of fluoxetine hydrochloride in D_2O at 25 °C

Sample	[H]/[G]	H-2'	H-3'	H-4'	H-5'	H-6'	$\Delta\delta\text{H-5'}/\Delta\delta\text{H-3'}$
A	1.40	0.054	-0.038	0.022	-0.291	0.028	7.66
B	1.05	0.057	-0.048	0.023	-0.310	0.020	6.46
C	0.88	0.057	-0.050	0.021	-0.308	0.017	6.16
D	0.45	0.059	-0.056	0.022	-0.319	0.009	5.70
E	0.40	0.057	-0.059	0.020	-0.315	0.009	5.34

Negative values indicate upfield shift.

more or less remained unchanged because concentration of the guest was kept constant.

3. Discussion

CDs have played major role in the development of supramolecular chemistry. Most of the CD related studies involve structural elucidation of the inclusion complex besides the energetics of the complex formation. The results of these studies suggest that the mode of complexation of the guest with CDs, involve insertion of the less polar (non-polar) portion of the guest into the CD cavity. They act as one site ligand meaning that the penetration mostly takes place from the wider rim side of cavity. Though these observations are mainly based on the studies of α -CD complexes but have mostly been found true for β -CD complexes. In the case of larger CDs the entry of guest may take place from either side, as in the case of γ -CD [1].

NMR spectroscopy has proved to be the most useful technique for the study of β -CD inclusion complexes because of its sensitivity [9]. The host–guest interactions are clearly reflected in changes on various NMR spectral parameters. The structure of an inclusion complex is determined by an energetic balance between the maximal inclusion of non-polar portion of guest into the β -CD cavity and optimal mutual orientations of host and guest to permit interactions of polar portions of the guest with β -CD or solvent.

Demarko and Thakkar first observed the variations in chemical shifts of H-3' and H-5' of β -CD in the presence of various substrates and inferred that inclusion in cavity has taken place since these protons are situated inside the cavity [10]. This type of evidence has since been widely collected. The development of new NMR techniques has further contributed to better understanding of structures of CD complexes [11]. A typical structural inference is that if only H-3' signal undergoes a shift in the presence of a substrate then cavity penetration is shallow, a deep penetration results in H-5' shift also [12]. The magnitude of shift changes for H-3' and H-5' have been shown to be related to the stability of inclusion complex; higher values of $\Delta\delta_{H-3'}$ and $\Delta\delta_{H-5'}$ meaning higher stability of the complex. Moreover, the ratio for the induced shift changes for these protons, $\Delta\delta_{H-5'}/\Delta\delta_{H-3'}$, has been correlated to the depth of inclusion of the guest into the CD cavity [13].

Contrary to the upfield shift changes experienced by the protons situated inside the CD cavity, all the protons of the guest, generally, show downfield shift changes on complexation and not only the proton/s included in the CD cavity which has been attributed, besides the shielding effect by the cavity, to other factors like changes in the microenvironment and guest conformational changes upon complexation. The downfield shift changes in protons of the guest are smaller in magnitude compared to CD protons and have mostly been neglected though these may be useful, using advanced NMR techniques, in identification of the part of the guest that is included in the CD cavity [11].

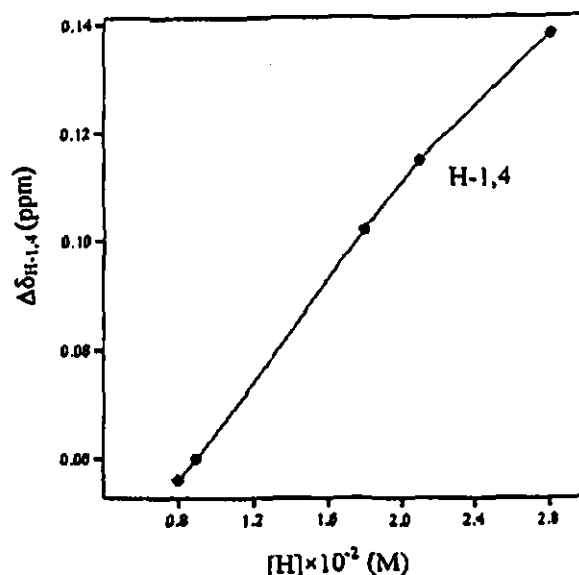


Fig. 2.

Remarkable upfield shift variations observed in the resonances of H-3' and H-5' of β -CD in the presence of fluoxetine hydrochloride clearly indicate the inclusion of aromatic part of the guest into the CD cavity due to hydrophobic interaction, in analogy to earlier studies [5,6,10]. As expected, most of the guest protons exhibited concomitant downfield shifts which increased with increasing [H], exhibiting type of saturation curvature (Fig. 2). A modified Hildebrand–Benesi plot of H-1,4 of trifluoromethyl substituted aromatic ring in the form of $[H]/\Delta\delta$ vs. [H] gave excellent linear fits (Fig. 3) which supports 1:1 complex formation [14]. The higher magnitude for $\Delta\delta_{H-3'}$ compared to $\Delta\delta_{H-5'}$ values may be due to either the penetration of aromatic guest from the narrow rim of β -CD [15] or deep penetration of the guest from wider side [13]. The observations that protons belonging to both the aromatic rings exhibit shift changes (though the chemical

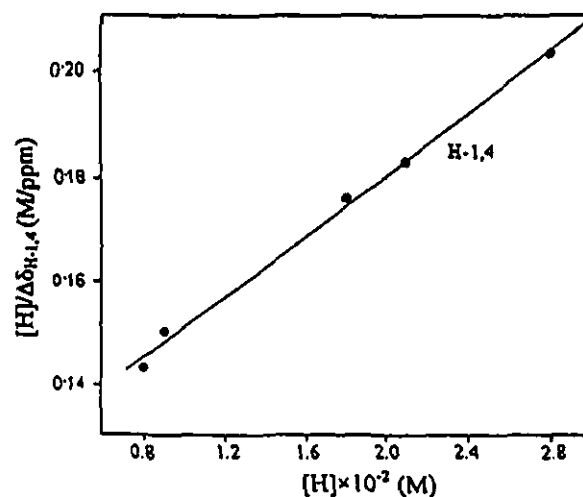
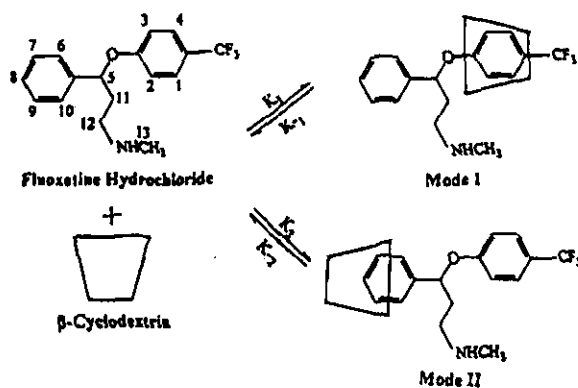


Fig. 3.

shift changes of phenyl ring could not be calculated because of their complexity), taking into account the 1:1 stoichiometry of the complex, indicates that two equilibria may exist in solution. The separation of signals of H-5 of two enantiomers in the presence of β -CD (Fig. 1), may be attributed to the fact that diastereomeric complexes formed from two enantiomers are structurally different from each other.

To have an insight into the mode and depth of penetration, confirm the existence of multi-equilibria in solution and detailed structure of complex/es, ROESY experiment was performed on a mixture of fluoxetine hydrochloride- β -CD. The presence of strong cross peaks between H-2,3 and H-1,4 of trifluoromethyl substituted aromatic ring with H-3' and H-5' of β -CD and relatively weak cross peaks between H-3' of β -CD and phenyl ring protons of guest (Fig. 4), confirmed the presence of two equilibria in solution. Several topologies may arise for each aromatic ring because entry may occur through either the larger or smaller rim of β -CD and the penetration may be either shallow or deep. The 2DROESY spectrum confirms binding mode-I for CF_3 -substituted ring with β -CD in which the guest enters from wider rim and penetrates deep so that the $-\text{CF}_3$ group protrudes outside the cavity and interacts with 6'-OH. This assumption is supported by the fact that H-1,4 showed upfield shifts in the presence of β -CD, which may be explained in terms of close proximity of these protons with fluorine in free state while in complexed state these F atoms may form H-bonds with 6'-OH, thus weakening the interaction between F and H-1,4. In this mode, these H-1,4 come in sizable contact with only H-5' while H-2,3 of guest are in the proximity of both H-3' and H-5' of β -CD. The cross peaks between phenyl ring protons with only H-3' of β -CD, which is near the wider rim, support the mode-II of various probable topologies meaning the pen-



Scheme 1.

etration of the phenyl ring is also from wider side but only a part of the ring enters the cavity. The deep penetration would bring some of the phenyl protons near the H-5'. A schematic representation of the inclusion equilibria is shown in Scheme 1. The dissociation constant, K_d , of the complex was calculated to be $1.4 \times 10^{-2} \text{ M}$ by a least-square fitting procedure from the plots (Figs. 2 and 3) [14,16].

In conclusion, ^1H NMR and 2DROESY spectroscopy of mixtures of fluoxetine hydrochloride and β -CD in D_2O at 25°C revealed the existence of two 1:1 diastereomeric inclusion complexes formed by the penetration of aromatic rings of guest into the β -CD cavity from wider rim side and that CF_3 -substituted ring penetrates deep while inclusion of phenyl ring is shallow. The dissociation constant, K_d , for the complex was determined.

Acknowledgements

The authors are thankful to Mr. Nikhil Deva, Cadila Pharmaceuticals for providing the gift sample of pure fluoxetine hydrochloride. Thanks are also extended to Professor Petri Turhanen, Department of Chemistry, University of Kuopio, Finland, for helping in obtaining NMR spectra.

References

- [1] M.L. Bender, M. Komiyama, *Cyclodextrin Chemistry*, Springer-Verlag, New York, 1978 p. 1.
- [2] J. Szejtli, in: *Cyclodextrin Technology*, Kluwer, Dordrecht, 1998, pp. 450.
- [3] J. Szejtli, Medicinal applications of cyclodextrins, in: *Medicinal Research Review*, vol. 14, 1994, pp. 364.
- [4] D. Duchene, C. Vaultier, F. Glomot, Cyclodextrins, their value in pharmaceutical technology, *Drug Dev. Ind. Pharm.* 12 (1986) 2193.
- [5] S.M. Ali, A. Maheshwari, F. Asmat, Complexation of roxatidine acetate hydrochloride with β -cyclodextrin: NMR spectroscopic study, *Pharmazie* 8 (2004) 653–655.
- [6] S.M. Ali, F. Asmat, A. Maheshwari, NMR spectroscopy of inclusion complex of D-(–)-chloramphenicol with β -cyclodextrin in aqueous solution, *IL. Farmaco* 59 (2004) 835–838.

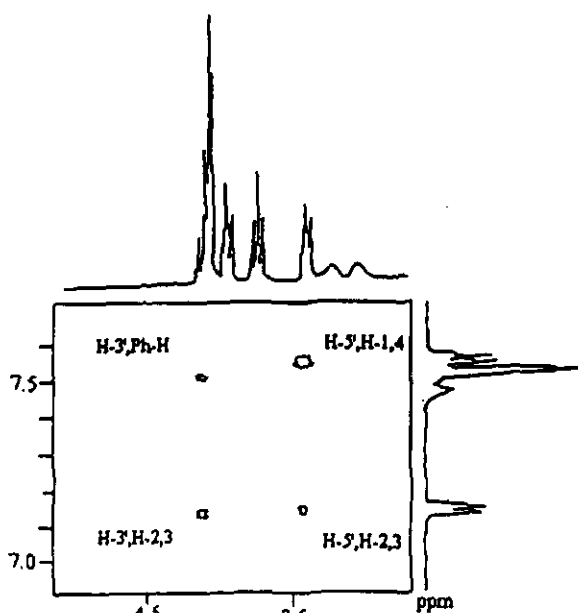


Fig. 4.

- [7] J.W. Stewart, F.M. Quitkin, P.J. McGrath, J. Amsterdam, M. Fava, J. Fawcett, et al., Use of pattern analysis to predict differential relapse of remitted patients with major depression during 1 year of treatment with fluoxetine or placebo, *Arch. Gen. Psychiatry* 55 (1998) 334–343.
- [8] D. Salvatierra, C. Jaime, A. Virgili, F. Sanchez-Ferrando, Determination of inclusion geometry for the β -cyclodextrin/benzoic acid complex by NMR and molecular modeling, *J. Org. Chem.* 61 (1996) 9578–9581.
- [9] H.-J. Schneider, F. Hacket, V. Rudiger, H. Ikeda, NMR studies of cyclodextrins and cyclodextrin complexes, *Chem. Rev.* 98 (1998) 1755–1786.
- [10] P.V. Demarco, A.L. Thakkar, Cyclohepta-amylose inclusion complexes. A proton magnetic resonance study, *J. Chem. Soc. Chem. Commun.* (1970) 2–4.
- [11] D. Neuhaus, M. Williamson, in: *The Nuclear Overhauser Effect in Structural and Conformational Analysis*, VCH, Publishers, New York, 1989, pp. 1.
- [12] R.J. Bergeron, R. Rowan, The molecular disposition of sodium *p*-nitrophenolate in the cavities of cycloheptaamylose and cyclohexaamylose in solution, *Bioorg. Chem.* 5 (1976) 423–436.
- [13] M.V. Rekharsky, R.N. Goldberg, F.P. Schwarz, Y.B. Tewari, P.D. Ross, Y. Yamashoji, Y. Inoue, Thermodynamic and nuclear magnetic resonance study of the interactions of α - and β -cyclodextrin with model substances, phenethylamine, ephedrine, and related substances, *J. Am. Chem. Soc.* 117 (1995) 8830–8840.
- [14] Z.H. Qi, V. Mak, L. Diaz, D.M. Grant, C. Chang, Molecular recognition: α -cyclodextrin and penicillin V inclusion complexation, *J. Org. Chem.* 56 (1991) 1537–1542.
- [15] T. Nakajima, M. Sunagawa, T. Hirohashi, K. Fujioka, Studies of cyclodextrin inclusion complexes. I. Complex between cyclodextrin and benzocyclopentadiene in aqueous solution, *Chem. Pharm. Bull. (Tokyo)* 32 (1984) 400–483.
- [16] R.J. Bergeron, M.A. Channing, G.J. Gibelly, D.M. Pitor, Disposition requirements for binding in aqueous solution of polar substrates in the cyclohexaamylose cavity, *J. Am. Chem. Soc.* 99 (1977) 5146–5151.

2 Complexation of Fluvastatin Sodium with β -Cyclodextrin: NMR 3 Spectroscopic Study in Solution

4 SYED MASHHOOD ALI^{1,*}, SANTOSH KUMAR UPADHYAY¹, ARTI MAHESHWARI¹
5 and MAMORU KOKETSU²

6 ¹Department of Chemistry, Aligarh Muslim University, 202 002, Aligarh, UP, India; ²Division of Instrumental
7 Analysis, Life Science Research Center, Gifu University, 501-1193, Gifu, Japan

8 (Received: 16 November 2005; in final form: 30 January 2006)

9
10 **Key words:** β -cyclodextrin, fluvastatin sodium, inclusion complex, NMR spectroscopy

11 Abstracts

12 ¹H NMR spectroscopic study of fluvastatin sodium (FLU), β -Cyclodextrin (β -CD) and their mixtures confirmed the
13 formation of FLU/ β -CD inclusion complex in solution. The stoichiometry of the complex was determined to be 1:1
14 and the overall binding constant (K_s) was calculated to be 340 M^{-1} . Two dimensional COSY, ROESY and DEPTO
15 experiments were performed for the unambiguous assignment of aromatic proton resonances and it was found that
16 two isomeric forms of FLU are present in solution. It was confirmed with the help of ROESY spectral data that
17 only F-substituted aromatic ring penetrates the β -CD cavity and there is chiral differentiation by the β -CD as one of
18 the isomer binds more strongly, which is indicated by the intensity of correlation peaks. The mode of penetration of
19 the guest into the β -CD cavity was also established and structure of the complex has been proposed.
20

21 Introduction

22 Fluvastatin sodium (FLU) helps in preventing heart
23 disease, angina, stroke and heart attacks by reducing the
24 cholesterol and certain other fatty substances in the
25 blood [1, 2]. It is a competitive inhibitor of 3-hydroxy-3-
26 methylglutaryl-coenzyme A (HMG-CoA) reductase and
27 lowers the overall blood cholesterol as well as blood
28 LDL (bad) cholesterol levels, which is responsible for
29 coronary artery diseases. FLU is light sensitive and its
30 solubility in water is 1.91 mg/ml.

31 Complexation of pharmaceutical compounds with
32 cyclodextrins (CDs) leads to altered physicochemical
33 properties of the guest. Inclusion complexes of phar-
34 maceutical compounds with CDs, therefore, have been
35 extensively studied and utilized to improve the solubility
36 [3], dissolution rate [4] and bioavailability of poorly
37 water-soluble drugs [5] and other desirable properties [6].

38 CDs are cyclic oligomers of α -D-glucose linked
39 through glycosidic α -1,4-bonds resulting in the forma-
40 tion of doughnut shaped molecules having one rim
41 (narrow) lined with n primary hydroxyl groups while
42 the other rim (wider), lined with $2n$ secondary hydroxyl
43 groups. The H-3', H-5' and glycosidic oxygen are
44 located inside the cavity which is relatively hydrophobic.
45 The CDs, therefore, act as hosts for a variety of non-
46 polar molecules [7–9].

NMR spectroscopy is an important tool to study CD
inclusion complexes [10]. ¹H NMR spectra of mixtures
of CD and guest molecule are recorded and changes in
the chemical shifts ($\Delta\delta$) of both the host as well as guest
are studied. The formation of the inclusion complex is
indicated by highfield shift changes in the CD protons
situated inside the cavity, namely H-3' and H-5' and
downfield shift changes in the guest protons. The
chemical shift change data can be used for the deter-
mination of stoichiometry, binding constant and mode
of penetration of the guest into the CD cavity. ROESY
[11] spectroscopy is particularly useful in the study of
inclusion complexes. NOE correlation peaks observed
between the protons of the included part of the guest
and β -CD cavity protons give direct evidence for the
formation of inclusion complex and provide very useful
information regarding the structure of the complex.

We are interested in the study of the inclusion com-
plexes of pharmaceutical compounds with β -CD in
solution [12, 13] and report herein our results on the
detailed NMR spectroscopic study of β -CD–fluvastatin
sodium complexation.

Results and discussion

All the ¹H NMR spectra were recorded on a Varian
Mercury Plus-300 instrument while COSY, DEPTO
and ROESY experiments were performed on a JEOL

* Author for correspondence. E-mail: smashhoodali@yahoo.com

73 α -400 MHz instrument in D₂O at room temperature and
 74 the chemical shift values (δ) are reported in ppm. No
 75 external indicator was used and HDO signal at 4.800
 76 was used as internal reference throughout this work. ¹H
 77 NMR spectra for five samples of FLU and β -CD with
 78 β -CD:FLU molar ratios ranging from 0.2 to 1.1 were
 79 recorded. The concentration of the FLU was kept constant
 80 at 1.6×10^{-2} M while that of β -CD was varied.
 81 Distinct peaks for bound and free form of the FLU were
 82 not observed indicating the rapid exchange of FLU
 83 between free and bind state on the NMR time scale.

84 ¹H NMR spectra of mixtures of FLU and β -CD
 85 displayed highfield shift changes in the H-3' and H-5' of
 86 the β -CD, located inside the cavity, while insignificant
 87 shift changes were observed for other β -CD protons.
 88 Expansions of part of the spectra showing β -CD protons,
 89 in the presence as well as in the absence of FLU,
 90 are shown in Figure 1. These high field shift changes in
 91 the β -CD cavity protons can only be explained in term
 92 of ring current effect of aromatic ring penetrating
 93 the β -CD cavity and thus confirm the formation of
 94 FLU β -CD complex, in analogy to previous studies [10,
 95 14]. This is also supported by concomitant downfield
 96 shift changes in the aromatic protons of the FLU in the
 97 FLU β -CD mixtures compared to pure FLU [14].

98 To clearly establish whether both or either of the two
 99 rings is involved in complexation, an unambiguous
 100 resonance assignment of FLU protons was required. A
 101 cursory examination of the aromatic region of the FLU
 102 spectra, in the presence as well as in the absence of

β -CD, points to the presence of two isomeric forms of
 103 FLU. The COSY, ROESY and DEPTO experiments
 104 were performed on a mixture of FLU/ β -CD, which
 105 proved very helpful in resonance assignment of the two
 106 isomers. Only the signals for the F-substituted aromatic
 107 ring appeared separately for the two isomers. The dou-
 108 blelet at 7.5634 ($J=6.88$ Hz) was assigned to the H-1 of
 109 one of the isomers. This signal showed cross peak, in the
 110 COSY spectrum, with the multiplet at 7.1048 suggesting
 111 that H-2 of one isomer is merged with H-3, 5 signal.
 112 It was confirmed by the DEPTO experiments that
 113 signals for the protons of the two aromatic rings are
 114 merged in the multiplet at 7.1048. The doublet at 7.3876
 115 ($J=6.88$ Hz) and triplet at 6.9742 ($J=6.88$ Hz) were
 116 assignable to H-1 and H-2, respectively, of the other
 117 isomer. The cross peaks between signals at 6.9742 and
 118 7.1048, observed in COSY spectrum, may arise due to
 119 the interaction between H-2 and H-3. Expansions of the
 120 COSY (Figure 2) and DEPTO (Figure 3) spectra of a
 121 FLU/ β -CD mixture, showing aromatic regions, support
 122

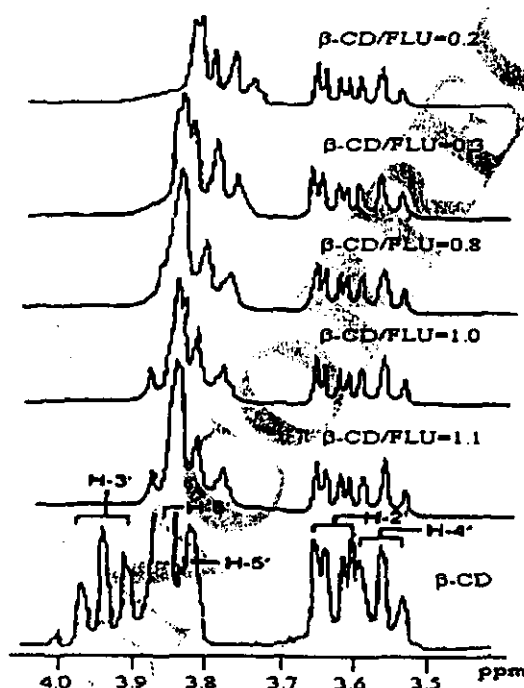


Figure 1. Partial ¹H NMR spectra (300 MHz) showing upfield shift of the β -CD protons with increasing amount of FLU.

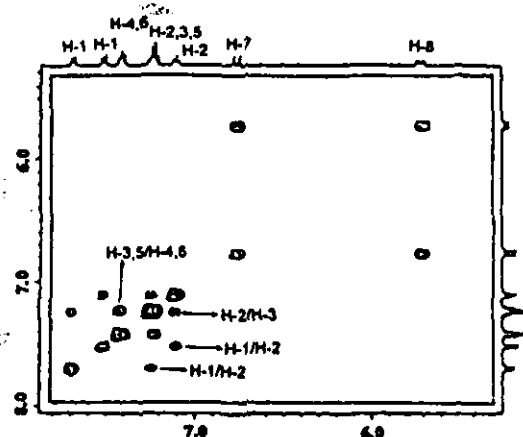


Figure 2. Part of the COSY spectrum (400 MHz) of a mixture of FLU and β -CD showing interaction of aromatic protons.

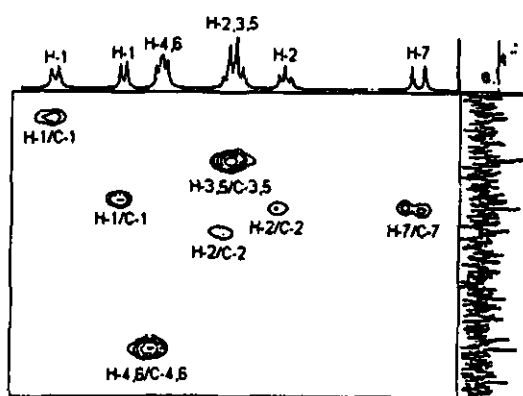


Figure 3. Part of DEPTO spectrum showing that H-2 is merged with H-3, 5.

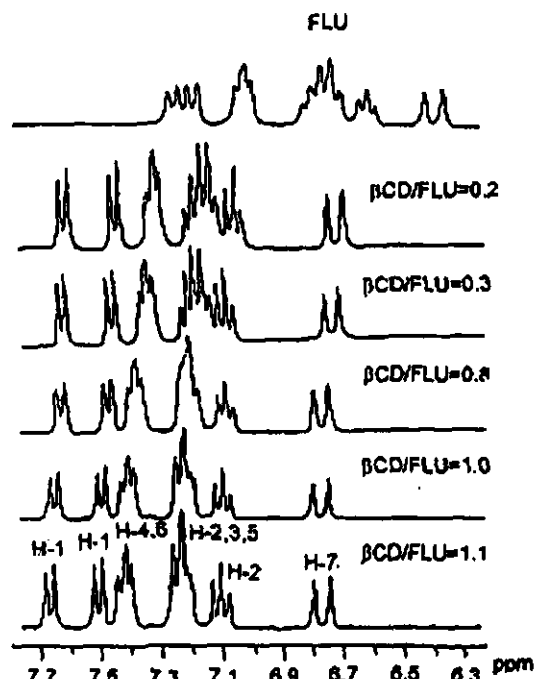


Figure 4. Partial ^1H NMR (300 MHz) spectra showing downfield shift of aromatic protons in the presence of β -CD.

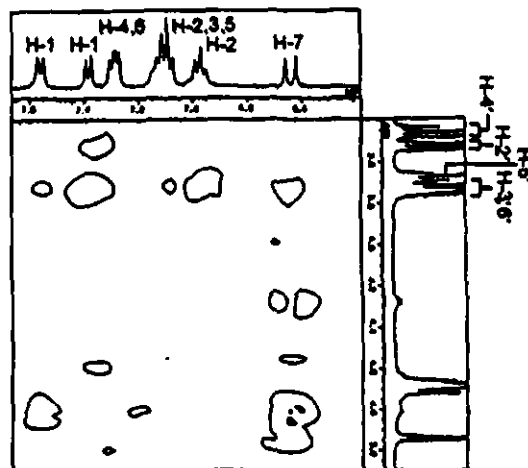


Figure 6. Expanded region of ROESY spectrum of β -CD/FLU mixture showing cross peaks of F-substituted aromatic ring protons with β -CD cavity protons.

the β -CD cavity show downfield shift changes upon complexation with CDs [15].

The stoichiometry of the complex was determined using Scott's modification [16] of Benesi-Hildebrand equation. In Scott's equation

$$[\text{CD}]_0 / \Delta\delta_{\text{obs}} = [\text{CD}]_0 / \Delta\delta_c + 1 / K_a \Delta\delta_c$$

where $[\text{CD}]_0$ is the molar concentration of the CDs, $\Delta\delta_{\text{obs}}$ is the observed chemical shift difference for a given $[\text{CD}]_0$ concentration, $\Delta\delta_c$ is the chemical shift difference between a pure sample of complex and the free component at the saturation. The plot of chemical shift changes ($\Delta\delta$) for the FLU protons against $[\beta\text{-CD}]$ in the form of $([\beta\text{-CD}]/\Delta\delta)$ versus $[\beta\text{-CD}]$ gave excellent linear fit (Figure 5) confirming 1:1 stoichiometry for the complex. The slope of the plot of $[\text{CD}]$ is thus equal to $1/\Delta\delta_c$ and the intercept with the vertical axis to $1/K_a \Delta\delta_c$, allowing the estimation of K_a . The binding constant (K_a) was determined to be 340 M^{-1} .

In order to clearly establish the structure of the complex, ROESY experiment was performed on a

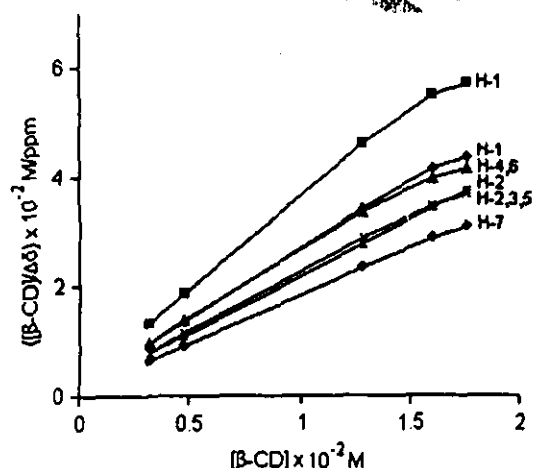


Figure 5. Scott's plot showing 1:1 stoichiometry of the β -CD/FLU complex.

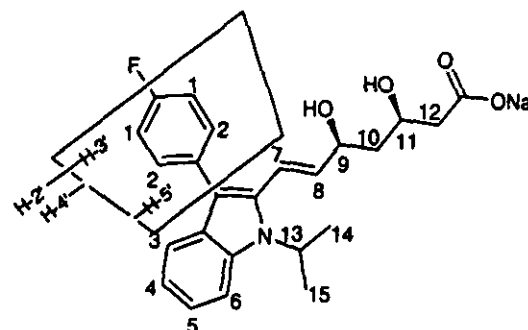


Figure 7. Plausible structure of the 1:1 β -CD/Fluvastatin sodium complex.

FLU/ β -CD mixture under spin lock conditions. The result is displayed in Figure 6; a set of cross peaks connects H-3' and H-5' resonances of β -CD to the signals for the F-substituted aromatic ring. The signals for the other aromatic ring did not show any cross peaks with β -CD cavity protons confirming beyond doubt the penetration of only F-substituted aromatic ring into the β -CD cavity. The intensity of the cross peaks for the two isomers was not identical suggesting chiral differentiation by the β -CD cavity [17]. Moreover, the H-1 of the F-substituted aromatic ring also showed cross connection peaks with H-2' and 4' protons, which are situated near the wider rim. This implies that aromatic ring penetrates deep from the narrower rim side [18] and H-1 is located near wider rim in the complex. The plausible structure for the FLU/ β -CD complex is shown in Figure 7.

In conclusion, the detailed NMR spectroscopic study of FLU in solution in the presence of β -CD confirmed the formation of 1:1 inclusion complex resulting by the penetration of the F-substituted aromatic ring into the β -CD cavity from narrower rim side. FLU is a mixture of two isomers and β -CD seems to play a role in their chiral differentiation by favourably binding to one of the isomers. The structure for the β -CD-fluvastatin sodium complex has been proposed.

Acknowledgements

Fluvastatin sodium was very kindly provided by Morepen Laboratories Ltd., India. We are also thankful to Dr. L.S. Mombaswala, Sophisticated Analytical

Instrument Facility, IIT, Mumbai, for his help in obtaining some of the data.

Reference

1. M.H. Davidson: *Am. J. Med.* 96, S41 (1994).
2. T.K. Peters, J. Jewitt-Harris, M. Mehra, and E.N. Mulatti: *Am. J. Hypertens.* 6, S346 (1993).
3. M.T. Esclusa-Diaz, M. Gayo-Otero, M.B. Perez-Marcos, and J.L. Villa-Jato: *Int. J. Pharm.* 142, 183 (1996).
4. A.A. Obaidat, S.M. Matalgah, and J. Al-Jarrah: *Acta Pharm.* S2, 9 (2002).
5. J.J. Torres-Labandeira, J. Blanco-Mendez, and J.L. Villa-Jato: *STP Pharm. Sci.* 4, 235 (1994).
6. J. Szejtli: *Medicinal Research Review* 14, 264 (1994).
7. M.L. Bender, and M. Kondo: *Cyclodextrin Chemistry*, Springer Verlag, New York (1971), pp. 1.
8. W. Saecker: *Angew. Chem. Int. Ed. Engl.* 19, 344 (1980).
9. J. Szejtli: *Cyclodextrin Technology*, Kluwer, Dordrecht (1988), pp. 450.
10. H.J. Schneider, E. Hackel, V. Rudiger, and H. Ikeda: *Chem. Rev.* 98, 1755 (1998) (and references cited therein).
11. E. Butkus, J.C. Martins, and U. Berg: *J. Inclusion Phenom.* 26, 209 (1996).
12. S.M. Ali, and A. Maheshwari: *Bull. Kor. Chem. Soc.* 26, 2061 (2005).
13. S.M. Ali, F. Asmat, and A. Maheshwari: *IL Farmaco* 59, 835 (2004).
14. M.M. Rekhani, R.N. Goldberg, F.P. Schwarz, Y.B. Tewari, P.D. Ross, Y. Yamashoji, and Y. Inoue: *J. Am. Chem. Soc.* 117, 8830 (1995).
15. F.B. Veiga, C.M. Fernandes, and R.A. Carvalho: *Chem. Pharm. Bull.* 49, 1251 (2001).
16. R.L. Scott: *Rev. Trav. Chim.* 75, 787 (1956).
17. T. Kitae, T. Nakayama, and K. Kuno: *J. Chem. Soc. Perkin Trans. 2*, 207 (1998).
18. E. Estrada, I. Perdomo-López, and J.J. Torres-Labandeira: *J. Org. Chem.* 65, 8510 (2000).